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**FEATURE PRESENTATIONS
EMISSIONS AND EFFLUENTS
ENVIRONMENTAL TECHNOLOGIES
PROGRAM
PREVENTION, TREATMENT AND
REMEDIAATION
TECHNOLOGIES/BIOTECHNOLOGY**

**THE MULTI-MEDIA APPROACH:
Integrated Environmental
Protection**

**November 25 & 26, 1991
Four Seasons
Inn on the Park
Toronto, Ontario
Canada**



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TECHNOLOGIES/BIOTECHNOLOGY

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Ontario, Canada

Introduction

Environment Ontario holds its annual Technology Transfer Conference to report and publicize the progress made on Ministry-funded projects. These studies are carried out in Ontario Universities and by private research organizations and companies.

The papers presented at the Environmental Research: 1991 Technology Transfer Conference are published in two volumes of conference proceedings corresponding to the following sessions:

- VOLUME I: FEATURE PRESENTATIONS
 ENVIRONMENTAL TRANSPORT AND FATE
 ENVIRONMENTAL EFFECTS
 ENVIRONMENTAL MANAGEMENT OPTIONS
- VOLUME II: FEATURE PRESENTATIONS
 EMISSIONS AND EFFLUENTS
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 PREVENTION, TREATMENT
 AND REMEDIATION
 TECHNOLOGIES/BIOTECHNOLOGY

Volume II is comprised of presentations given during Session D, Session E, Session F and Session G of the conference, as well as all Feature Presentations.

For reference purposes, indices for all sessions may be found at the front of both volumes.

For further information on any of the papers, please contact either the authors or the Research and Technology Branch at (416) 323-4649 or 323-4573

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FEATURE PRESENTATIONS

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PAPER NOT AVAILABLE AT TIME OF PRINT

Environmental Transport and Human Exposure: A Multimedia Approach in Health-Risk Policy

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Introduction

In his treatise *Air, Water, and Places*, the ancient-Greek physician Hippocrates demonstrated that the appearance of disease in human populations is influenced by the quality of air, water, and food; the topography of the land; and general living habits. This approach is still relevant and, indeed, the cornerstone of modern efforts to relate public health to environmental factors. What has changed is the precision with which we can measure and model these long-held relationships. Today, environmental scientists recognize that plants, animals, and humans encounter environmental contaminants via complex transfers through air, water, and food and use multimedia models to evaluate these transfers. In this talk, I explore the use of multimedia models both to examine pollution trends and as a basis for characterizing human health risks and ecological risks. The strengths and weaknesses of the approach are discussed.

I begin with a review of multimedia models—how they came about and where they are going. I highlight areas of success, areas of weakness, and areas that need much more work—such as plant-soil-air modelling. Next, I look at recent efforts to integrate multimedia models with exposure models to develop a more complete picture of human exposure to environmental contaminants. This is followed some ideas about the formal treatment of uncertainty in multimedia models and how this could bring about a better understanding of their precision and accuracy. Finally, I will discuss some new directions for multimedia model research.

Multimedia Models

Efforts to assess human exposure from multiple media are not particularly new. The need to assess human exposure to global fallout in the 1950's led rapidly to a framework that included transport both through and among air, soil, surface water, vegetation, and food chains. Efforts to apply such a framework to nonradioactive organic and inorganic toxic chemicals have been more recent and have not as yet achieved the level of sophistication extant in the radioecology field.

The first widely used multimedia compartment models for organic chemicals were the "fugacity" models developed by Mackay (1979, 1991) and Mackay and Paterson (1981, 1982). Cohen and Ryan (1985) introduced the concept of the

multimedia compartment model as a screening tool with the MCM model. At the Lawrence Livermore National Laboratory (LLNL), we have developed a multimedia screening model, called GEOTOX (McKone and Layton, 1986), which was one of the earliest multimedia models to explicitly address human exposure for nonradioactive contaminants.

In a multimedia model we lump major components of the environment into homogeneous subsystems or compartments that can exchange mass with other adjacent compartments. Quantities, concentrations, or fugacities within compartments are described by a set of linear, coupled, first-order differential equations, which can be solved under steady-state or dynamic conditions. A compartment is described by its total mass, total volume, solid-phase mass, liquid-phase mass, and gas-phase mass. Mass flows among compartments include solid-phase flows, such as dust suspension or deposition, and liquid-phase flows, such as surface run-off and ground-water recharge. The transport of individual chemical species among compartments occurs by diffusion and advection at the compartment boundaries. Each chemical species is assumed to be in chemical equilibrium among the phases within a single compartment. However, there is no requirement for equilibrium between adjacent compartments. Decay and transformation processes (such as radioactive decay, photolysis, biodegradation, etc.) are treated as first-order, irreversible removals. The compartment structure we are using for assessing the regional impacts of toxic substances in air, water, and soil is illustrated in Figure 1.

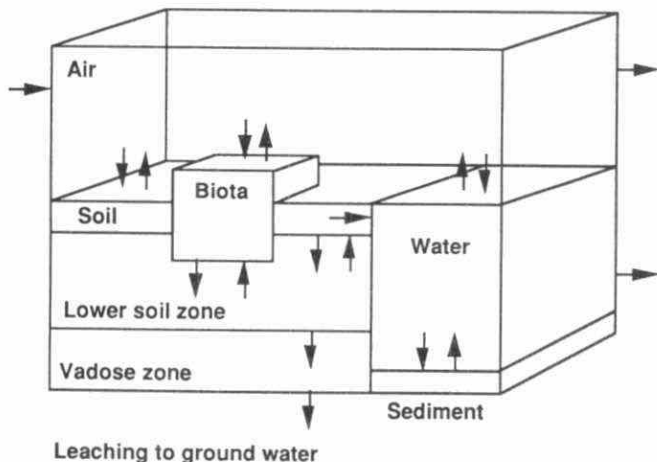


Figure 1. An illustration of mass exchange processes modeled in a seven-compartment environmental transport and transformation model.

Multiple Pathway Exposures

Human exposures to environmental contaminants result from contact with contaminated soils, water, air, and food as well as with drugs and consumer products. The extent of such exposures depends on (1) human factors and (2) the concentrations of a chemical in contact media. Human factors include all behavioral, sociological, and physiological characteristics of an individual that directly or indirectly affect his or her contact with the substances of concern. The principal output of an exposure assessment is a quantitative estimate of contact, expressed as mass of chemical per kg body weight per day. Exposures should be estimated for specific routes (i.e., inhalation, ingestion, and dermal uptake) because route-specific uptake, distribution, and metabolism are accounted for in pharmacokinetic models that could be used in risk assessments.

An early approach for systematically assessing multiple pathway exposures to nonradioactive environmental contaminants is the exposure commitment method (ECM) (Bennett, 1981). Exposure commitments (i.e., contaminant concentration in human tissue) are calculated from transfer factors that are the ratios of the steady-state concentrations of contaminants in the compartments of an exposure pathway. An exposure commitment is determined by multiplying the transfer factors of a given pathway, for example, air→plants→livestock→diet. This method has been applied to organic chemicals and metals. Applications of the ECM are retrospective and depend on measured concentrations of the substances in the different compartments to estimate transfer factors.

McKone and Daniels (1991) have developed an approach that explicitly allows for the estimation of exposures to a substance based on either measured or predicted concentrations of the substance in contact media. Pathway-exposure factors (PEFs) are used to link chemical concentrations in multiple environmental media to human exposure by route—inhalation, ingestion, uptake. Specifically, each PEF numerically translates a chemical concentration in each of the primary environmental media (air, water, and soil) into exposure rates (in mg/kg-d) for each route of exposure. Incorporated into each PEF is information on human physiology and life style, as well as data describing pollutant behavior in food chains or in microenvironments, such as indoor air. In contrast to the ECM, this approach is prospective. This type of prospective approach has been used by Chetty (1991) to assess the impacts of benzene and pyrene in the Los Angeles basin.

Uncertainties

At best, mathematical models only approximate real systems, and therefore their predictions are inherently uncertain. The need to address human exposure in a multimedia framework brings with it a need to characterize the uncertainty in human exposure models and the combined uncertainty in exposure and dose/response models. In characterizing uncertainty in exposure models, three key issues should be considered: (1) uncertainty in predicting the relationship between sources of contaminants and concentrations in the accessible environment; (2) uncertainty in quantifying pathway-exposure factors (PEFs) that relate

environmental concentrations to levels of exposure; and (3) the important contributions to the combined uncertainty in environmental dispersion and pathway-exposure factors.

Future Directions

In their efforts to construct new multimedia-exposure models, environmental scientists should recognize the importance of being both comprehensive and realistic in developing exposure scenarios. In addition to an emphasis on comprehensive human exposure modelling and formal treatment of uncertainties, there are four other areas that, I believe, will be important for the future of multimedia models—(1) formal validation studies (2) improved air-plant-soil models, (3) ecological risk assessment, and (4) ecological economics. Little real progress with multimedia models will occur until there is a coordinated effort to validate a multimedia model both with measured data and with other models. The recent literature reveals new understanding about the transfer of contaminants among soil, air, and terrestrial plants and this information needs to be incorporated into the next generation of multimedia models. Also, multimedia models could provide valuable input to the process of calculating ecological risk, but so far the literature in this area is incomplete. Finally, the newly established field of ecological economics (Costanza, 1989), which seeks to address broad-scale relationships between ecosystems and economic systems, is an area well-suited match to the multimedia approach, but the courtship has yet to begin.

Acknowledgements

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Dioxin Analysis State-of-the-Art: the study of multimedia contaminants

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Introduction

Studies of the sources, transport and fate, and effects of the chlorinated dibenzo-p-dioxins (dioxins) and dibenzofurans (furans) are among the most difficult in environmental research. Central to these investigations are effective analytical methods that can quantify ultra-trace concentrations of dioxins/furans in a wide range of complex sample types. Developments in science as applied to toxic environmental contaminants essentially are the story of the dioxins/furans, as there is a close parallel between advances in analytical methods and advances in the environmental sciences. Because of the numerous studies of dioxins/furans in air, water, soil, industrial emissions, and in human and animal tissue, the dioxins and furans may be considered the first chemicals studied as true multimedia contaminants.

The History of Dioxin and Furan Contamination

Environmental contamination episodes. In 1957 millions of broiler chickens in the U.S.A. died after ingesting toxic compounds in feed fats. It was not until 1966 that one of the toxic compounds was identified as 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (1,2,3,7,8,9-H₆CDD) by x-ray crystallography. In the 1960's attention was focused on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) produced as a trace side-product in the manufacture of chlorophenols. Since chlorophenols were used as starting products in the manufacture of a number of industrial chemicals including the widely used herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,3,7,8-TCDD contamination of the environment became widespread. Incidents such as the spraying of Agent Orange in Vietnam from 1962-1970; the industrial accident at Seveso, Italy in July, 1976; and improper waste disposal at many Missouri sites during the early 1970's that resulted in the 1982 evacuation of the town of Times Beach, received intensive public scrutiny and are now well-documented. The presence of these chemicals in old chemical dump sites such as the Love Canal has also been highly publicized. In 1968 and 1979, cases of human poisoning from accidental ingestion of PCBs occurred in Japan and Taiwan,

respectively. The presence of some chlorinated dibenzofurans in the PCBs was thought to be the principal agent responsible for the adverse health effects observed. Other studies showed that furans are formed during the uncontrolled burning of PCBs after a series of PCB-filled capacitor fires occurred in several different countries. One of the most publicized capacitor fires took place in an office building in Binghamton, N.Y. in 1981. Furans are also found in chlorophenols and related industrial chemicals. The above issues are all described in references 1-4.

Other sources of dioxins and furans. In 1977 dioxins were found in the fly ash from municipal waste incinerators in The Netherlands (5). The following year, this finding was confirmed in fly ash from Ontario incinerators (6). In 1979 the New York State Department of Health announced the finding of 2,3,7,8-TCDD in two Lake Ontario fish. The Ontario Ministry of the Environment (MOE) set up a special laboratory to study this issue in October, 1980. By 1981 a data base of a few hundred analyses of 2,3,7,8-TCDD in fish was developed separately by Ontario and by New York State. Since the finding of 2,3,7,8-TCDD in fish showed that some waterborne route of contamination existed, MOE also began testing Lake Ontario surface water and drinking water. MOE's detection limits were 10 parts per trillion (ppt) in fish and 1ppt in 1L water samples (7). In September, 1985, the U.S. EPA found elevated levels of 2,3,7,8-TCDD in a few fish sampled in areas near Pulp & Paper mills. Intensive investigations by MOE during 1985-86 confirmed that dioxins and furans were contaminants of mills that employed chlorine in the bleaching process (8). In 1989 the Ontario petroleum refining industry was found to be a source of dioxins and furans during the regeneration of spent catalyst material used in the catalytic reforming process (9). Other sources were characterized in the late 1980's. These included automobile exhaust (10), metal recovery facilities (11), steel mills (12), and other metallurgical industries (13).

Enough is now known to suspect any combustion process as being a possible source of dioxins and furans. This was stated a decade ago by the Dow Chemical Company as the "Trace Chemistries of Fire" hypothesis (14). Little work has been performed on natural combustion sources such as forest fires, but a recent MOE study has shown that dioxins and furans are formed under forest fire conditions (15).

Dioxins and Furans as Multimedia Contaminants

All of the above studies show that humans can be exposed to dioxins and furans from air, water, and soil exposure pathways. A recent investigation showed that food commodities accounted for up to 95% of the exposure of Ontario residents to these chemicals (16). However, this exposure only amounted to 23% of the total tolerable daily intake (TDI) of 2,3,7,8-TCDD (Toxicity Equivalents or TEQ, see below) of 10 picograms (pg) per kg body weight (kg bw). All routes of exposure must be included in any model used to estimate exposure of humans to dioxins and furans. The multimedia exposure model used for Ontario has been described (17).

The basis for the multimedia approach is straightforward: once the maximum exposure level for a chemical has been determined, this quantity must be apportioned between all routes of exposure to protect human health. In this way, the combined exposure permitted by the separate guidelines would not exceed the TDI. Since food is the principal exposure pathway for Ontario residents, most of the tolerable daily dose would be apportioned to this pathway when determining guidelines. The net effect of the dose allocation exercise is that the guidelines set for any one exposure pathway will be much lower than if the entire daily tolerable dose was considered. The multimedia approach thus provides an effective means to protect human health, and in a sense symbolizes the interconnectedness of the ecosystem. The scientific advances in multimedia standard setting for the dioxins/furans can be applied to other environmental contaminants.

Challenges in the Analysis of Dioxins

The need for low detection limits. There are several reasons why parts-per-trillion (ppt) and parts-per-quadrillion (ppq) detection limits are required:

- ☐ the LD₅₀ for 2,3,7,8-TCDD is about 1.0 µg/kg bw in the most sensitive species
- ☐ dioxins are hydrophobic and lipophilic, and consequently have high bioconcentration factors - therefore low detection limits are required to identify and track sources, especially in aquatic systems
- ☐ for the study of dioxin levels in human and animal tissues, sample sizes may be very small
- ☐ long-term chronic effects from low-level exposures are not well understood

The need to separate dioxins from other organics in the sample. This task is the most challenging and the success with which sample cleanup is performed will determine the detection limits, precision, and accuracy that are attained. Since dioxins may be present in the final sample extract at low picogram quantities, they may be "swamped out" by other organics that are present in quantities thousands of times greater. The sophisticated gas chromatograph-mass spectrometer (GC-MS) systems employed for the final separation and detection steps of the analysis, although highly selective, cannot achieve ppt and ppq detection limits unless presented with a "clean" sample in which virtually all other organics have been removed. It should also be remembered that highly contaminated samples where dioxin levels may be significant are precisely those samples where other chlorinated organic compounds - structurally similar to the dioxins and therefore potential interferences - are also likely to be present.

The need to separate the dioxins from each other. Of the 210 possible chlorinated dioxin/furan structures, only 17 are thought to be of toxicological significance. These are the ones with chlorine substitution at the 2,3,7 and 8-positions of the basic structure. The other "non-toxic" dioxins and furans are therefore interferences for the 17 "toxic" compounds. Schemes have been developed to relate the relative toxicity of each 2,3,7,8-substituted dioxin/furan with that of the most toxic: 2,3,7,8-TCDD. For example, octachlorodibenzo-p-dioxin (OCDD) is considered to be 1000 times less toxic than 2,3,7,8-TCDD, while 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-P₅CDF) is thought to be one-half as toxic. Therefore "toxicity equivalent", or TEQ factors can be applied to the concentrations of OCDD and 2,3,4,7,8-P₅CDF in a sample to determine their "2,3,7,8-TCDD-toxicity equivalent" concentrations. The OCDD concentration is multiplied by 0.001, while the 2,3,4,7,8-P₅CDF concentration is multiplied by 0.5. By doing this for all 17 of the 2,3,7,8-substituted dioxins/furans and then summing up the "2,3,7,8-TCDD-toxicity equivalent" concentrations, a total TEQ value is obtained that is very useful for regulatory and risk-assessment activities. However, this exercise is only effective if the "toxic" and "non-toxic" dioxins/furans can be separated from each other. Since the 210 dioxins/furans are so similar in structure to each other, this is not an easy task.

Dioxin Analysis State-of-the-Art

The challenges described above, although substantial, have largely been solved. High resolution GC-MS systems are available that can routinely detect 100 femtograms or less of 2,3,7,8-TCDD. Detection of 10 fg has been reported. Complex cleanup schemes have been developed that effectively isolate the dioxins/furans from potential chemical interferences in the most difficult sample types. The keys to successful cleanup are the number of separation steps employed, and the care and skill provided by the analyst. By using a series of gas chromatographic columns, and/or a combination of gas chromatography and high performance liquid chromatography, definitive separation of all 2,3,7,8-substituted dioxins and furans from all non-toxic dioxins/furans is possible.

Detection limits in real samples of ppt or better have been reported in samples of biota (including food) and soils; parts-per-quadrillion levels are routinely attained in 1L water samples; and detection of 0.01 pg/m³ dioxin in ambient air is commonplace for the best 10-20 laboratories world-wide. A precision of 20-30% relative standard deviation (%RSD) for such work is possible if conditions and calibration standards are carefully controlled. However, it is not unusual to observe RSDs of 50-100% for dioxin in difficult samples when measurements are made near the method detection limit.

Where Do We Go From Here?

In a recent NATO survey, over 100 laboratories world-wide were identified that have some level of dioxin capability. Just 15 years ago, only about a dozen could make this claim. Of course, not all of these laboratories have the same level of expertise. There are probably no more than 20 labs who can boast state-of-the-art capability for isomer-specific determination of the 17 toxic dioxins/furans in virtually any matrix at ppt to ppq concentrations. One thing that even the top labs have not been able to do is to reduce the cost of analysis. It is still such a specialized area requiring highly trained staff and sophisticated, expensive instrumentation that private labs charge from \$1,000 to \$2,000 per analysis. Reduced cost for dioxin determinations would allow many more determinations to be performed. This would not only benefit environmental surveys (more locations monitored) and quality control (more replicates and round-robins), but would help multimedia investigations that are very sample-intensive (because all exposure pathways must be investigated).

Some researchers feel that some PCBs exhibit sufficient toxicity that they should be included with the 17 toxic dioxins/furans for the calculation of total TEQs. To do this would require even lower detection limits for the dioxins and furans. Since PCBs in sufficient concentrations are serious interferences for dioxin determinations, including some of them in the TEQ calculation would present a difficult challenge.

In the past 20 years, our knowledge of the formation, transport and fate, effects, and remediation of toxic environmental contaminants have greatly advanced because of dioxin/furan research. These advances would not have been possible without the parallel development of sophisticated analytical methodologies.

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THE MULTIMEDIA APPROACH AND POLLUTION PREVENTION:
WHAT DO WE KNOW? WHAT DO WE NEED TO KNOW?

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ABSTRACT

The theme of this conference is "The Multi-media Approach: Integrated Environmental Protection" and the theme of this session is "Environmental Management Options". This paper places these themes in the broader context of the economic literature, to reviews the implications of that literature for the conference themes, and considers directions for future economic research. Special emphasis will be placed on the potential role of experimental economics in evaluating environmental policy.

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THE MULTIMEDIA APPROACH AND POLLUTION PREVENTION:
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OUTLINE

This paper places the concept of Integrated Environmental Protection into the economic literature, reviews the implications of that literature for the environmental management, and considers directions for future economic research. Special emphasis will be placed on the potential role of experimental economics in evaluating environmental policy.

I Introduction

The theme of this conference is "The Multi-media Approach: Integrated Environmental Protection" and the theme of this session is "Environmental Management Options". This paper is intended to place these themes in the broader context of the economic literature, to review the implications of that literature for the conference themes, and to consider directions for future economic research. Special emphasis will be placed on the potential role of experimental economics in evaluating environmental policy.

It will be useful to define our terms. Environmental Management Options are institutions governing the interaction between the economy and the environment. On the one hand we can attempt to prescribe the exact conditions and quantities in which industrial, municipal and domestic wastes can be discharged to the natural environment. This is the "command-and-control" option often denounced in the economic literature. On the other hand we can attempt to manipulate the information people have and the incentives they face so as to encourage them to change their interactions with the natural environment. This is the so called "incentive based" option frequently advocated in the economics literature. Of course we could attempt some mixture of these somewhat opposing approaches. I shall have a considerable amount to say about this choice in the middle section of this paper.

The terms "multi-media approach", "integrated environmental protection", and "pollution prevention" are somewhat vaguer. In practice, it seems we are using a multi-media approach if we recognize that any waste discharged to any one medium (for example toxic wastes discharged to the air during incineration) may eventually wind up in a second medium (water, for example) and possibly reincorporated into human activity through even a third medium, such as the fish we eat. We are not using a multi-media approach if we speak about air, water, or soil pollution by itself. Thus calling for a multi-media approach to environmental protection is effectively a claim that exclusive focus on any one of air, land or water is going to lead to important errors in public policy.

"Integrated environmental protection", it would appear to me, has essentially the same content. I take it that our environment is protected in an integrated manner if we simultaneously control discharges to air, water and land. "Pollution Prevention", on the other hand, seems to refer more to the choice between "end-of-pipe treatment" of residuals and process innovations designed to reduce residual discharges.

II Multi-media Approaches in the Context of the Economic Literature

Stressing integrated environmental protection is consistent with a large body of literature in environmental economics. Two strands in the literature seem particularly relevant. These are the materials balance approach to environmental economics and the rapidly expanding literature on economics of sustainable development.

A The Materials Balance Approach

The materials balance approach dates back to Kneese and forms the introductory portion of all environmental texts. The relationship between the economy and the natural environment can be pictured in various ways. The natural environment contributed physical materials and intangible services to the human economy. The economy discharges wastes to the natural environment where they may affect the original flow of materials and services. All materials extracted from the environment are ultimately returned to it as residuals. Focus on this fact immediately leads to the problem of multi-media contamination, since the control of discharges to any one medium must necessarily alter discharges to another.

Recycling and pollution prevention can reduce the residual load placed on the environment, but they have labour, capital and natural resource requirements themselves. We must select the appropriate level of recycling and pollution prevention, while recognizing that reduced demands on the environment may have an opportunity cost in terms of reduced incomes and consumption of other goods.

B The Economics of Sustainable Development

There is a large and growing literature on the economics of sustainable development. Much progress has been made in giving economic content to the idea of sustainable development. Here I wish to argue that efficiency should be a central concern of environmental quality management, and that it is too often neglected. In discussing policies for sustainable development, the literature also makes useful distinctions among general policies with environmental effects, framework policies concerning the environment, and issues surrounding project evaluation.

1 Sustainable Development and Efficiency

The literature distinguishes development from economic growth. Economic growth involves increases in measured income such as GNP. Development refers to indicators of human welfare. It is argued that growth cannot be sustainable, but that development can.

2 Policies for Sustainable Development

Stimulating work on environmental policy has occurred at all three levels. At the level of general policies, new developments in National Accounting are leading to the incorporation of depreciation of the Natural environment into the national accounts. At the level of environmental policy, the great debate concerns the choice between command-and-control regulation and incentive based regulation. Discussion of this is postponed to the next section. At the level of project evaluation, sensible suggestions have been made for the incorporation of environmental concerns, but general practice in the field lags far behind.

III The Choice of Regulatory Regime

This section focuses on the choice between Command and Control and incentive based regulation. The paradox is the great enthusiasm evidenced for market based schemes in the Green Plan and elsewhere while the assessments of the US market based schemes are surprisingly pessimistic. Several explanations for the limited success of these schemes are examined.

We may react to the presence of environmental problems in several ways: we may ignore them, we may try to solve them through the private law system, or we may try to regulate. In the last case we are faced with the choice between command and control and incentive based regulation. The case for incentive based regulation seems strong: incentive based regulations should minimize the cost of attaining any given level of environmental control and should provide strong incentives for the development of new control technologies. But the experience in the United States, especially with air emissions trading, has been only a modest success. Far fewer trades are made than had been expected. Explanations offered include excessive restrictions on trades (levels, sequential trades, the need to meet standards at every point (see Merriwell, Atkinson and Tietenberg), and political difficulties in getting the concept accepted. This last literature suggests concerns about the income distributional consequences of emission trading schemes is important.

IV The Contribution of Experimental Economics

This section suggests that some of the difficulties encountered in promoting and testing the ideas of incentive based regulation can be answered by laboratory experiments. Economics is not usually considered to be an experimental science. There is a growing literature, however, that tests the effectiveness of economic institutions in a laboratory setting. The

systematic examination of institutions for allocating environmental rights is only beginning. There are, however, a number of experiments that show great promise. Further work in this area is advocated.

V Conclusion

This paper has attempted to place the themes of environmental management options and the multi-media approach to pollution prevention into the context of the economic literature. It has argued that great progress has been made in suggestions for incorporating environmental concerns into economic decisions, but that practice lies well behind theory. The debate between command-and-control and incentive-based regulation is unresolved. The newly developing field of experimental economics shows substantial promise in helping us to understand the nature of the debate.

D3 Costs and Benefits of Improved Environmental Control
- Some Case Histories at INCO; W.C. Ferguson, INCO
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PAPER NOT AVAILABLE AT TIME OF PRINT

F2 A Consumer Perspective to Waste Prevention and Reduction; R. Lotzkar, Environmentally Sound Packaging Coalition and Consumers' Association of Canada, Vancouver, British Columbia

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G7 Close Encounters of the Immune Kind: Antibody-Based
Tests for Environmental Chemicals; B. Ferguson,
ImmunoSystems Inc., Scarborough, Maine, U.S.A.

PAPER NOT AVAILABLE AT TIME OF PRINT

VOLUME II
SESSION D
EMISSIONS AND EFFLUENTS
VERBAL PRESENTATIONS



Modelling the influence of topography on dense gas dispersion II: Algorithm development

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1 INTRODUCTION

Topography, in the form of general slope, isolated hills, or more complex terrain, will alter or divert a dense gas cloud or plume. The topography may enhance plume dilution and divert the plume away from regions of elevated terrain; or, the dense plume may be channelled into valleys or low-lying areas and then be protected from the diluting influence of the ambient flow. These important effects are not included in the models currently used to predict the dispersion of a dense gas cloud for risk assessment or emergency management.

The overall objective of the study is the development of methods for incorporating the effects of topography in numerical models of dense gas dispersion. Phase I of the project, reported previously, consists of an extensive review of the existing theory and experimental results applicable to the problem. Phase II of the project consists of the development of algorithms for incorporating the effect of topography into integral (box) models of dense gas dispersion. This paper outlines the development of algorithms, based on the previous review (Ramsay & Britter 1990a, 1990b, 1991a).

2 ALGORITHM DEVELOPMENT

Previous publications from this project (Ramsay & Britter 1990a, 1990b, 1991a) have shown that it should be possible to incorporate the effects of topography on dense gas dispersion within the box model formulation. These results have shown that these effects can be represented principally by: a modified advection velocity; and, a modified entrainment relationship. The treatment of other subsidiary aspects of the problem which are also necessary to provide a practical implementation of these ideas is outlined.

2.1 Relevance of slope

It is important to determine whether terrain effects should be considered or not. Fay & Ranck (1983) suggest a criterion, for an isolated release, that there will be no slope effect if

$$u_*^2 > \frac{g_0 Q_0}{\pi R^2} \sin \theta \quad (1)$$

this may be re-written as

$$\frac{g_0 \bar{z}}{u_*^2} < \frac{1}{\sin \theta} \quad (2)$$

where θ is the slope. This appears to be a reasonable criterion and might also be applicable to continuous plume releases.

2.2 Advection Velocity

It is shown in Ramsay & Britter (1990a, 1991c) that the advection velocity of the cloud is modified by topography.

There are two possible approaches to a modified advection velocity relationships in the model: a momentum equation approach; and, a modified advection velocity approach. Each of these approaches is discussed in the following sections.

2.2.1 Momentum Equation Approach

One possible approach for incorporating the effect of topography is to develop a momentum equation for a puff or plume. However, it is considered that this would obscure important aspects of the development and interpretation of the resulting model.

2.2.2 Modified Advection Equation Approach

Analysis and experimental results indicate that the advection velocity (for a plume) could be modelled as

$$U_a = U_{ambient} + U_d = U_{ambient} + C(g_m \bar{z})^{1/2} \quad (3)$$

where C is a function of the slope and the last term is the downslope velocity. The coefficient C is a weak function of slope and may be obtained from experimental results in the absence of any ambient wind. In these cases the flow adopts a constant value of

$$C = \frac{U_d}{(g_m \bar{z})^{1/2}} \quad (4)$$

due to a local balance between a downslope force and retardation due to skin friction and/or entrainment. The downslope velocity U_d is given by

$$\frac{g_m \bar{z}}{U_d^2} \cos \theta = \frac{E + C_D}{S_2 \tan \theta - \frac{1}{2} E S_1} \quad (5)$$

where

$$S_1 \approx 0.25 \quad S_2 \approx 0.75 \quad (6)$$

Solution of this equation requires an expression for the entrainment E . We shall use

$$E = 1.2 \times 10^{-3} \theta \quad (7)$$

for continuous or time-varying releases and the same expression with the coefficient 4.0×10^{-3} for instantaneous releases. These entrainment expressions are known to be valid for large (say $\theta > 5^\circ$) slopes and have been used for slopes down to 6×10^{-3} degrees. At small slopes the use of

$$E = \frac{U_t}{U_a} = C_1 \left(\frac{g_m \bar{z}}{U_a^2} \cos \theta \right) C_D^{3/2} \quad (8)$$

brings in the explicit dependence of entrainment on surface roughness.

Thus we must solve three equations to determine the entrainment E , the downslope velocity U_d and the advection velocity U_a .

We shall use the larger of the estimates of E based on θ and the estimate of E based on C_D . We shall use this result in continuous, time-varying and instantaneous releases. Note that this produces a reduced uphill advection velocity and an increased downhill advection velocity.

2.3 Modified Entrainment Equation

In dealing with entrainment we note two effects:

- (i) Entrainment due to the difference between the buoyant downslope motion and the ambient motion. This difference is just $C(g'z)^{1/2}$. We appeal here to the basic entrainment relation from Ellison & Turner (1960) and others that an additional entrainment velocity

$$u_t = (U_a - U_{ambient}) (1.2 \times 10^{-3} \theta) \quad (9)$$

where θ is the slope in degrees which leads to

$$u_t = C(g'_m \bar{z})^{1/2} (1.2 \times 10^{-3} \theta) \quad (10)$$

should be included for plumes and the same expression with the coefficient 4.0×10^{-3}

$$u_t = C(g'_m \bar{z})^{1/2} (4.0 \times 10^{-3} \theta) \quad (11)$$

- (ii) A change in the surface generated turbulence. This might be expected to depend on the advection velocity. The proposal here is to model this using previous entrainment expressions but with u , modified to

$$u_s \frac{U_a}{U_{ambient}} \quad (12)$$

This is consistent with the small slope expression given above.

The correlation based on slope alone, i.e. (i), has, effectively, included (ii) albeit in an approximate way. Consequently we proceed in a manner consistent with the previous analysis and select the larger of the two estimates.

2.4 Reversing Flows

It is possible that the combined effect of topography and ambient wind will lead to a situation in which the motion of the cloud reverses. This situation is considered below for instantaneous and continuous releases.

2.4.1 Instantaneous Releases

These could move downslope and then upslope and this would be handled directly by the previous algorithms.

2.4.2 Continuous Releases

This case could be handled directly to allow for the reversing of the plume. However, we note that after the plume had reversed it would be interacting with the downslope plume. However, the downslope extent is limited by plume widening rather than by dilution. Consequently, the model would determine the width at the maximum downslope extent and then consider an undiluted, wide plume starting from this position.

2.5 Ambient Wind

When the ambient wind is not normal to the slope the model will consider the downslope velocity

$$C(g_m \bar{z})^{1/2} \quad (13)$$

in two components parallel and normal to the wind direction. The along wind component is used as before while the across wind component provides a normal velocity, the respective magnitudes providing the plume/puff direction. Entrainment would be treated as previously in two parts: and increased surface generated turbulence and an explicit slope dependent part.

2.6 Wind field determination

The topography will alter the wind field substantially. Three approaches are possible for estimating the altered wind field:

- i) Estimation of the maximum and minimum changes from analysis and experiment (e.g. Britter, Hunt & Richards 1984 and others) followed by interpolation. For example, Britter (1982) estimated that for flow over a hill of height h and length L (the half length to the half height, is about 25% of the overall length of the hill) there will be an increase in the local friction velocity u_* by

$$u_* \frac{h}{L} \left(\frac{U(L)}{U(\xi)} \right)^2 \quad (14)$$

where $U(z)$ is the incident ambient velocity profile and

$$\frac{1}{L} \ln \left(\frac{\xi}{z_0} \right) = 2\kappa^2 \quad (15)$$

where κ .

The decrease in u_* at the hill base will be similar. The velocity profile may be determined from the modified u_* , z_0 , L , H and ξ . More sophisticated estimates are possible.

- ii) Use specific codes (e.g. MS3DJH, FLOWSTAR or MSFD) for wind fields in complex terrain.
- iii) Specific codes might be used for a number of standard cases e.g. plateau to slope, plateau to slope to plateau etc., and a simple library formulated.

We will initially use the first approach.

3 RESULTS

Preliminary results have been obtained using these algorithms in the GASTAR dense gas dispersion model (Ramsay 1990). Figure 1 shows results for an instantaneous release similar to the Thorney Island trial 15 with no slope, and for upslope and down slope winds on a 5° slope. The radius and the concentrations are plotted as functions of the location of the cloud centroid. The upslope wind increased the radius of the cloud relative to the no slope case and the downslope wind decreased the radius. The concentration was increased relative to the no slope case for a downslope wind and decreased for an upslope wind. Figure 2 shows the results for a release similar to a Thorney Island test on a slope of 10° with the wind at 45° to the slope.

4 CONCLUSIONS

The results of the first two phases of this project have clearly indicated that the dominant effects of topography can be incorporated within the box model formulation of dense gas dispersion. The approach used involves a modified advection velocity and a modified entrainment relationship, together with subsidiary conditions required to provide a practical implementation of these ideas.

Preliminary results have shown good agreement between box model predictions using the GASTAR dense gas dispersion model and experimental results. The two remaining phases of this project will further explore the implementation of these algorithms in a dense gas dispersion model, for routine risk assessment and emergency management use and comparison tests with experimental data and more sophisticated model results.

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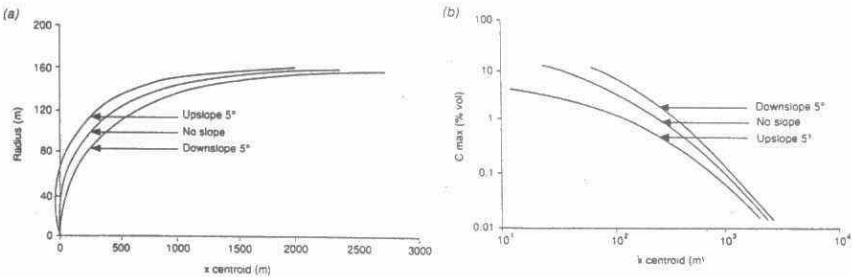


FIGURE 1 The effect of slope on Thorney Island trial 15 instantaneous release results. (a) radius (b) centreline concentration.

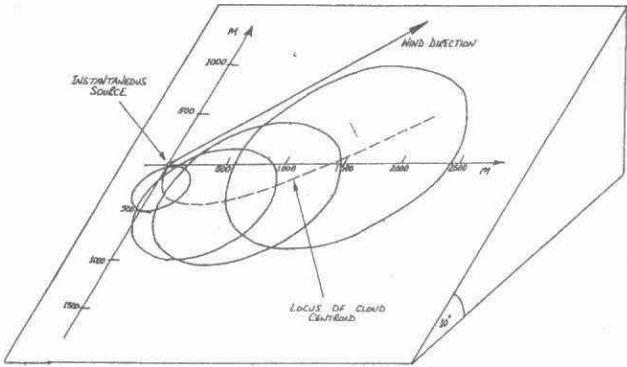


FIGURE 2 Effect of slope on an instantaneous release similar to the Thorney Island releases on a 10° slope with ambient wind at 45° to the slope. The cloud initially moves down the slope under the influence of the negative buoyancy, then dilutes and is driven up the slope by the ambient wind.

PRACTICAL APPLICATION OF FECAL COLIFORM (FC) TO STREPTOCOCCUS
FAECIUM SUBSP. CASSELLIFLAVUS (SC) AND BIFIDOBACTERIUM TO SC
RATIOS TO DETERMINE HUMAN AND ANIMAL SOURCES OF POLLUTION.

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INTRODUCTION

Fecal coliforms and Escherichia coli (EC) are routinely used as fecal pollution indicators. However, these organisms are found in both human and animal feces and therefore cannot be used as indicators of strictly human input. Many organisms pathogenic to humans are also found in animals; one example is Campylobacter jejuni (Manninen et al., 1982). Other pathogens, such as the hepatitis A virus (Shinjo, et al. 1980), are human-specific. Since these organisms can be transmitted via the fecal-oral route, it is important to be able to assess whether the pollution comes from a human or animal source.

Streptococcus faecium subsp. casseliflavus (SC) has been shown in earlier work to be present in animal but not in human fecal samples (Seyfried et al., 1990). Studies are currently underway to examine the feasibility of utilizing this organism in an EC:SC ratio model to predict the origin of fecal pollution.

Mossel proposed the use of bifidobacteria as indicators of fecal contamination in 1958 (D.A.A. Mossel, Abstr. 7th Int. Congr. Microbiol., 1958, p. 440); however, more work is necessary to determine their true potential. In principle, Bifidobacterium spp. have all the characteristics of an ideal indicator. For example, they are found in high densities in fecal material (Mitsuoka and Kaneuchi, 1977); they are incapable of surviving in the oxygenated extraenteral environment and therefore show promise as an indicator of recent fecal contamination (Oragui, 1982); and the possibility of developing a reliable test to determine human or animal sources of pollution exists (Cabelli, 1979; Resnick and Levin, 1981).

Our previous studies have shown that Bifidobacterium spp. and S. faecium subsp. casseliflavus can be recovered from sanitary and storm sewers (Seyfried et al. 1990). Methods are being developed to facilitate the isolation and characterization of these organisms and to study their rates of survival. This paper reports on a study assessing the use of these organisms to determine the impact of wildlife and other types of fecal pollution on the Kelso Conservation area.

METHODS

Sampling sites

The nine sampling sites were located on Sixteen Mile Creek in the Kelso Conservation area and in the City of Milton.

Sample collection

Surface water samples were collected in sterile bottles on a weekly basis. The samples were collected between 9:00 a.m. and 12:00 noon because our earlier studies showed that incident radiation between noon and 3:00 p.m. on sunny days caused a drop in bacterial levels (Seyfried, 1973). Processing in the lab was within 5h of collection.

Human and animal fecal specimens were also collected for analysis.

Isolation and characterization methods

Escherichia coli

Samples were filtered through 0.45 - μ m (pore size), 47 - mm - diameter type GN-6 membrane filters (Gelman Sciences, Ann Arbor, Michigan). The filters were placed on mTEC medium and incubated in plastic containers with bottles of ice at 44.5°C for 24 h. To test for urease production, the filters were transferred

to petri dishes containing pads saturated with urea. All yellow colonies were counted, after a 15 min incubation, as presumptive *E. coli*.

Streptococcus faecium subsp. casseliflavus

The isolation medium consisted of the m-Enterococcus agar (Difco) formulation without the addition of 2,3,5 - triphenyl tetrazolium chloride. Samples were membrane filtered and the filters were placed on the agar surface. Incubation was at 37°C for 48 h. The colonies appeared pale, lemon, or bright yellow in colour. For confirmation, filters were placed on plates of nutrient agar with 2% gelatin. Following incubation at 37°C for 1.5 h, the filters were removed and the plates were flooded with mercuric chloride solution (Mates, 1983). The target colonies are gelatinase negative as well as xylose and raffinose positive. Arginine hydrolysis and pyruvate tests were carried out on selected isolates.

Bifidobacterium spp.

The isolation medium employed was Lactobacillus - MRS (Difco) with 0.03% cysteine hydrochloride added. Appropriate dilutions of each sample were spread plated onto the agar surface. Plates were incubated at 37°C for 48 h in anaerobic jars containing an anaerobic gas pack (BBL 70304) and an indicator strip (BBL 70504). Typical bifido colonies appeared porcelain white (pearl-like), opaque, mucoid, concave, round, and entire.

Additional methods used to further classify and speciate the bacteria included the F6PPK (fructose-6-phosphate phosphoketolase) test (Scardovi, 1986) and the API Rapid CH test (API Laboratory Products Ltd., St. Laurent, Quebec).

Survival studies

E. coli C3000 was grown for 24h at 37°C in nutrient broth. A 1mL aliquot was added to 9 mL phosphate-buffer solution and this diluted culture was then placed in a flask with 90 mL of filter-sterilized farm pond water. Incubation

of each flask was at 0°C, room temperature, or 37°C. S. faecium subsp. casseliflavus was grown for 24 h at 37°C in Brain Heart Infusion broth (Difco) and diluted into pond water as described for E. coli. Samples were taken weekly and plated onto the selective medium noted previously for each organism.

RESULTS AND DISCUSSION

The analysis of human and animal fecal specimens showed that, in humans, E. coli (EC) densities averaged 4.03×10^6 per gram. To date, no S. faecium subsp. casseliflavus (SC) has been isolated from human feces; therefore, the calculated EC:SC ratio for human feces would be 4.03×10^6 . In comparison, analysis of over 30 horse fecal specimens yielded an average of 6.46×10^4 E. coli per gram and 6.23×10^4 S. faecium subsp. casseliflavus per gram. Thus the ratio of EC:SC for horses is close to 1.0. Other animals studied also had approximately equal densities of EC and SC.

Preliminary field studies were conducted in the Kelso Conservation Area to assess the feasibility of using the EC:SC ratio as a means of determining whether pollution input is of human or animal origin.

The data obtained from HFI and M2, two of the nine sampling sites shown in Fig. 1, are presented here for comparative purposes. The HFI location has animal input from wildlife. Raccoon and deer feces collected from the area had S. faecium subsp. casseliflavus levels ranging from 1.0×10^5 to 3.8×10^6 CFU per gram. Escherichia coli densities in the fecal specimens ranged from 1.7×10^5 to 1.4×10^8 . The M2 site is representative of human pollution input because of outflow from the Milton sewage treatment plant.

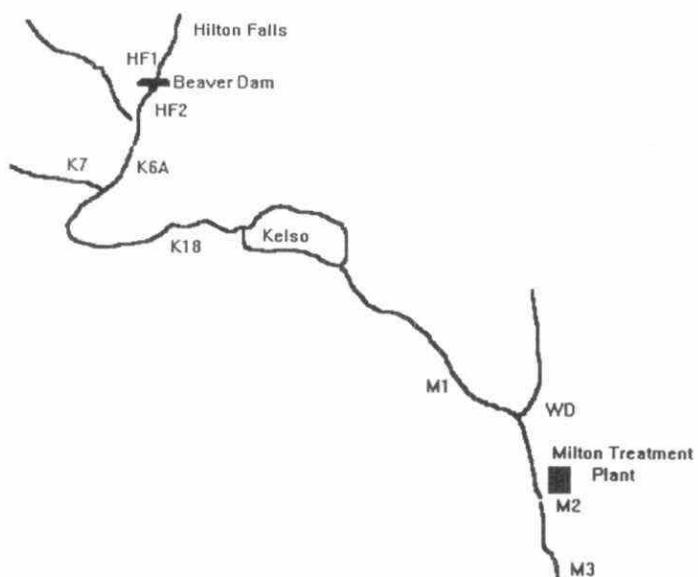


FIG. 1. Sampling locations on Sixteen Mile Creek.

Table 1. Comparison of *E. coli* (EC) and *S. faecium* subsp. *casseliflavus* (SC) levels and ratios at the HFI site, which has animal impact, and at the M2 sewage treatment plant location.

Date (1991)	HFI		Sampling Site		
	EC	: SC ^a	EC	: SC	M2
June 25	141	: 11 (12.8)			ND
July 2	122	: 29 (4.2)	11	: 0 (11)	
July 9		ND	39	: 0 (39)	
July 16	400	: 24 (16.7)	96	: 0 (96)	
July 23		ND	23	: 0 (23)	
July 30	1000	: 101 (9.9)	2400	: 0 (2400)	
Aug. 6		ND	198	: 0 (198)	
Aug. 13	185	: 20 (9.3)	80	: 10 (8)	
Aug. 20		ND	20	: 0 (20)	

^a CFU/100 mL of *E. coli* and *S. casseliflavus*

() = Calculated ratio

ND = Not determined

As may be seen from Table 1, apart from one sample collected on August 13, no *S. faecium* subsp. *casseliflavus* organisms were isolated from samples collected at the main outflow pipe of the Milton sewage treatment plant. This resulted in an average EC:SC ratio of 349.4 at this site which had a major human pollution input. The average EC:SC ratio of samples taken from the HFI site, where there was a wild animal pollution source, was 10.6. Although the detailed results are not presented here, the EC:SC ratios determined for the other sampling sites approximated those found at the HFI location. These seven sites received input from farm runoff and other animal sources.

A more precise assessment of the type of animal pollution input would be helpful in tracing, for example, runoff from different point sources. To determine if it is possible to identify an individual animal source, *S. faecium* subsp. *casseliflavus* isolates from a variety of animal fecal specimens were examined for their colony morphology and biochemical characteristics. Some differences between animals were noted. For example, the pigment and colony texture varied from a soft, lemon yellow colony for sheep to a bright yellow, dry and sticky colony for pigeons. Differences were also noted in the arginine hydrolysis and pyruvate reactions as shown in Table 2.

Table 2. Arginine hydrolysis and pyruvate reactions in *S. faecium* subsp. *casseliflavus* isolates from various animal sources.

Animal Source	Number of isolates	Arginine hydrolysis	Pyruvate
Horse	85	+ (100%)	- (96.5%)
Pigeon	7	+ (100%)	+ (57.1%)
Chicken	24	+ (100%)	- (100%)
Goat	6	+ (100%)	- (100%)
Goose (domestic)	18	+ (94.4%)	± (94.4%)
Goose (wild)	13	+ (100%)	- (100%)
Pig	1	+ (100%)	- (100%)
Turkey	2	+ (100%)	± (50%)
			- (50%)
Cow	7	+ (85.7%)	- (100%)
Camel	1	+ (100%)	- (100%)
Chipmunk	1	+ (100%)	- (100%)
Sheep	40	+ (60.0%)	- (97.5%)

As may be seen in the Table, the arginine hydrolysis reaction was positive in almost all cases. Some variation was observed for the pyruvate reaction and this will be investigated further.

Mundt (1961) reported on the frequent recovery of enterococci from plants. Sherman (1937) also suggested that these organisms occur commonly on plants. In this study, samples of grass, hay, carrots and lettuce were analyzed and it was found that S. faecium subsp. casseliflavus densities on these plants were in the 2.0×10^1 to 1.56×10^4 CFU per gram range. When the EC:SC ratios were calculated they ranged between 0.005 and 0.05. Although these organisms are readily isolated from plants, Mundt concluded that they are only temporary residents, disseminated by the action of wind and insects. Since the plant EC:SC ratios appear to be very low, their interference in pollution source determinations will be minimal.

The results of the survival studies carried out in farm pond water are presented in Table 3.

Table 3. Percent survival of E. coli and S. faecium subsp. casseliflavus in pond water at varying temperatures over a two month period.

Organism	Number of weeks	0°C	Room Temperature	37°C
<u>E. coli</u>	2	99.10%	180.55%	7.69%
	4	7.50%	111.11%	0.06%
	8	2.10%	0.14%	0.01%
<u>S. faecium</u> subsp. <u>casseliflavus</u>	2	94.25%	28.89%	48.12%
	4	83.91%	7.33%	9.37%
	8	44.83%	0.60%	0.68%

The data showed that E. coli died off more rapidly at 0°C and 37°C than did S. faecium subsp. casseliflavus. However, at room temperatures ranging from 20 to 25°C there was regrowth of E. coli. Other researchers (Bonde, 1977,

Evison and James, 1973) have shown that E. coli may be able to survive and regrow for extended periods in tropical habitats. Carrillo and coworkers (1985), for example, found that E. coli was able to regrow in the Mameyes River when the water temperature was 21 to 22°C. Although S. faecium subsp. casseliflavus survived longer than E. coli at temperatures of 0°C and 37°C, the regrowth phenomenon was not observed.

Table 4. Levels of Bifidobacterium spp. and E. coli per gram of human and animal feces.

Source	<u>Bifidobacterium</u> spp.	CFU per gram <u>E. coli</u>
Human	6.31×10^7	4.03×10^6
Chicken	1.28×10^8	7.09×10^7
Horse	1.56×10^5	6.46×10^4
Cow	1.02×10^7	2.21×10^7
Pig	2.57×10^6	3.66×10^7
Sheep	3.02×10^5	2.67×10^8
Goat	1.16×10^7	1.21×10^8
Rabbit	1.55×10^6	8.50×10^7
Goose	1.35×10^7	1.10×10^7
Turkey	5.17×10^8	5.39×10^7
Duck	9.28×10^7	1.06×10^8

As shown in Table 4, although human feces contain large numbers of bifidobacteria, high levels can also be recovered from domestic and wild animals. Beerens (1991) was able to isolate Bifidobacterium spp. from the domestic rabbit, horse, chicken, swallow, crow, domestic rat, dog, pig and fox as well.

Diet appears to play an important role in bifido colonization. Trovatelli and Matteuzzi (1976) studied the numbers and species of bifidobacteria present in the rumen of calves fed high-roughage and high-concentrate diets. They found that bifidobacteria were not detectable in a 10^{-3} dilution with the roughage diet, whereas with the high-carbohydrate concentrate ration their numbers were generally in the order of 10^8 to 10^9 per mL of rumen fluid. Bifidobacterium ruminale and B. globosum were the species most frequently isolated.

Our previous work (Seyfried *et al.*, 1990) showed that B. adolescentis and B. breve were the predominant species in human feces and sewage. Carrillo and co-workers (1985) also found that B. adolescentis was isolated most frequently (88.9% of the 105 isolates) from tropical freshwater samples, followed by B. angulatum (5%), B. infantis (5%) and Bifidobacterium spp. (unknown) (1.1%).

Mara and Oragui (1983) have suggested that sorbitol-fermenting bifidobacteria, such as B. adolescentis and B. breve, be used as human fecal pollution indicators. This proposal has merit but, due to the fact that B. adolescentis has been isolated from dogs and B. breve from chickens (Seyfried *et al.*, 1990), further studies are required.

CONCLUSION

The EC:SC ratio shows promise as a method of indicating human or animal sources of pollution.

Bifidobacterium species are present in high levels in human fecal material but high densities also occur in animal feces. More research is needed to determine if the species are human or animal specific.

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VOLUME II

SESSION D

EMISSIONS AND EFFLUENTS

POSTER PRESENTATIONS



GEOCHEMICAL CHARACTERIZATION OF METAL/PARTICULATE ASSOCIATIONS IN 3
OUTFALL TYPES TO THE DON RIVER. L.A. Warren* and A.P. Zimmerman, Department of Zool-
ogy, University of Toronto, Toronto, Ontario M5S 1A1.

Reductions of suspended solids and metals are important components in water quality remediation of the Toronto Area Watershed. Despite the fact that the majority of metals end up in association with particulates, solids reduction will not necessarily lead to trace metal reductions - particularly reductions in potentially bioavailable trace metals. Sediment geochemistry is of primary importance in determining trace metal partitioning; and depending on which geochemical fractions are involved (e.g. oxides, clays, organics), metals may be bound, only to be released again given changes in system chemistry. We have been evaluating trace metal transport by suspended sediments in the Don River over the last 3 years. There is a considerable amount of univariate deviation in these results which is not surprising given the myriad contaminant sources and the hydrologic regime of the Don. However, multivariate analysis of these data indicate that the geochemical phase of the particulates combined with information on dissolved salts e.g. Cl^- and Ca^{2+} , explain a large proportion of that variance. By examining specific types of discharge sources, the relative influence of those sources for *in situ* river patterns of trace metals/suspended sediment associations can be determined. Such a relationship may be a means of "fingerprinting" specific discharge sources to the Don. Prioritization of source abatements and the effectiveness of proposed remedial action plans can then be objectively evaluated. We are assessing the trace metal/suspended sediment associations of 3 outfall types on the Don (STP, "residential" and "industrial"). Outfall samples are sized, and metals (Cd, Cu, Zn and Pb), associated with 4 operationally defined sediment geochemical fractions (exchangeable, oxides, organics, and residue minerals) are extracted, and subsequently analyzed by flame atomic absorption.

MONITORING AIR POLLUTION SOURCES (MAPS)

MAPS Mobile Stack Emissions Monitoring Laboratory
Al Melanson
Gary Wong
Source Assessment & Technology Unit
Air Resources Branch
Ontario Ministry of the Environment
Toronto, Ontario

THE MAPS Mobile Laboratory (MAPS) is a 30' Orion bus fitted with continuous emission monitors and all associated equipment (sample conditioners, pumps and transport systems, data acquisition and processing systems, etc..) to continuously measure stack gas concentrations of oxygen, carbon dioxide, carbon monoxide, hydrocarbons, nitrogen oxides, sulfur dioxide, total reduced sulfur and hydrogen chloride. External devices to measure other emission factors such as opacity, stack temperature, and flow rates are also present.

The MAPS is utilized by the Air Resources Branch, Source Assessment & Technology Unit to conduct continuous emission measurements at stationary sources, perform audits on continuous emission monitoring systems, and to provide support to special emission characterization studies. The information gathered is essential in assessing stack monitoring systems' performance, efficiency of emission abatement equipment, and compliance with emission standards.

In the past year, MAPS has conducted source monitoring surveys at London Victoria Hospital's Energy from Waste plant to audit the performance of their continuous emission monitoring system, took part in the Ministry's Recycled Tire/Rubberized Asphalt project by providing continuous emission measurements of conventional and rubberized asphalt production at two Ontario asphalt plants, and assessed compliance with oxygen, total hydrocarbon, and carbon monoxide emission standards at the Smithville CWML Decontamination Site incinerator.

In order to meet the needs of its ever expanding mandate, the MAPS Mobile Laboratory is continually updated with state-of-the-art equipment to increase the unit's versatility, and to assure monitoring data accuracy and completeness. The MAPS Mobile Laboratory provides an essential service to the Air Resources Branch and the Ministry's Regional staff.

SOURCE CHARACTERIZATION OF EMISSIONS FROM RESIDENTIAL WOOD BURNING

Claude S. Davis* and D.M. Dougherty, Concord Environmental Corporation, 2 Tippet Road, Downsview, Ontario, M3H 2V2.

INTRODUCTION

The increased use of residential wood combustion in Ontario and several other areas of North America has resulted in adverse air quality impacts in some areas. A recent literature review of such impacts and of the legislative approaches directed at minimising such impacts, pointed to the need for the characterization of the organic and inorganic components in residential wood stove emissions from typical firewood and stoves used in Ontario. The source characterization study will be followed by a monitoring program designed to indicate the ambient air quality impacts of residential woodburning in selected communities in Ontario.

Most of the earlier work on source characterization focused on inorganic components and organic carbon. Recently, source profile information on organic components especially polynuclear aromatic compounds is being determined. The influence of stove type, fuel (wood) type and condition, and of woodstove operating conditions on source profiles represent formidable challenges in reliably characterizing source profiles. Source testing in the present study was designed to measure emissions during steady state burning using three stove commonly used in Ontario and using maple, birch and jackpine firewood - three of the commonly used firewoods in Ontario.

This presentation outlines the preliminary results of the source testing of emissions from three wood stoves (designated A, B and C) and three types of wood. The primary objective of the testing is to obtain source profiles (for organic and inorganic species) for three typical fire woods and wood stoves used in Ontario. Source testing was completed in mid-September and preliminary results for the organic components analyzed are discussed here. Analytical data for inorganic species were unavailable of the time of writing.

METHODS

The test facility was located at the University of Waterloo, Department of Mechanical Engineering. Well cured and dried firewood obtained from commercial suppliers was stored indoors at the test facility. A 4" flue was attached to each stove and the exhaust gases were vented through existing exhaust ducts in the laboratory. A sampling port was located 2 m from the base of the stove exhaust. A modified Ontario Ministry of the Environment (OME) Method 5 sampling train (NAPP Inc. Model 63) was used to obtain samples of emissions. Standard protocols were established to conduct each test to ensure consistent stove operation. For each test, the stove was allowed to reach a steady state condition representative of steady burning conditions. At least duplicate tests were conducted on each woodstove/wood combination.

All test materials (filters, solvents and sorbents) were analyzed prior to the initiation of tests. All target analyte compounds had acceptable blank levels. The sampling train consisted of a heat traced probe with a nickel plated stainless steel nozzle, and attached thermocouple and pitot tube. After the probe, the gas passes through a cyclone, a heated filter holder (glass fibre filter) and a sorbent module containing XAD-2 resin. Four impingers are downstream of the sorbent module; the first two impingers contained HPLC grade water, the third was empty and the fourth contained silica gel. The impingers are followed by a pump, dry gas meter and a calibrated orifice meter.

Proofs of the sampling train and blank filters also acceptable blank levels for all analytes. Single point non-isokinetic sampling was carried out for approximately 50 to 80 minutes to obtain a sample volume of 0.6 to 1 m³ (dry standard cubic metre (DSCM)). The precise sample volume for each run was calculated. Traditional measurements throughout the run of flue gas velocity, temperature, volume and pressure were made. Since the flow rates from wood stoves are so low, and because the majority of particulate emissions occur in the very fine size fraction (< 2.5 µm diameter), non-isokinetic sampling was considered adequate for meeting the objectives of this study. The particulate fraction was collected on a glass fibre filter and the semivolatile organics were collected on XAD-2 resin. Samples of stack gas were collected in tedlar bags for analysis of CO and CO₂.

The sampling train was analyzed as three fractions as follows. The rinse from the front half (nozzle, probe liner and cyclone) together with the first three impingers were combined and analyzed as the first fraction. The filter (one half) and the resin were analyzed separately as the second and third fractions. Each fraction was soxhlet extracted in dichloromethane, solvent exchanged in cyclohexane and then extracted with toluene and analyzed by GC/MS for 24 organic species including 16 PAH. The filter was analyzed by ICP for 21 elements.

RESULTS AND DISCUSSION

The total mass of each component in the sample was calculated by summing the masses in each of the three fractions analyzed. There were negligible amounts of analytes in the probe rinse and impinger fraction; the majority of the constituents were present in the resin. The negligible amount in the probe and impinger fraction indicates that most of the organic species are present on the vapour phase and that negligible amounts of organics are present in larger particles which would be deposited in the probe.

The concentration of each species was calculated from the total mass and the corrected (to standard dry gas volume at 25 °C and 1 atmosphere) sample volume. The source profiles are expressed as the ratio of each component relative to the concentration of benzo(e)pyrene.

Within each stove type the profiles were most consistent for the heavier PAHs - perylene, indeno(1,2,3,c-d)pyrene, dibenzo(a,h)anthracene, benzo(e) and benzo(a)pyrene. The lighter and more volatile PAH (anthracene, fluorene, phenanthrene) showed greater variability in their profiles. Stove B gave most consistent results even for the more volatile compounds.

The profiles for each wood type showed no major differences suggesting that wood types and stove types used have no dramatic influence on the source profiles for the components measured in this study.

CONCLUSIONS AND RECOMMENDATIONS

More detailed examination of test conditions and analysis of the inorganic data will take place before firm conclusions are drawn regarding the influence of stove type and wood on the source profiles.

VOLUME II

SESSION E

ENVIRONMENTAL TECHNOLOGIES PROGRAM

VERBAL PRESENTATIONS

ECO LOGIC



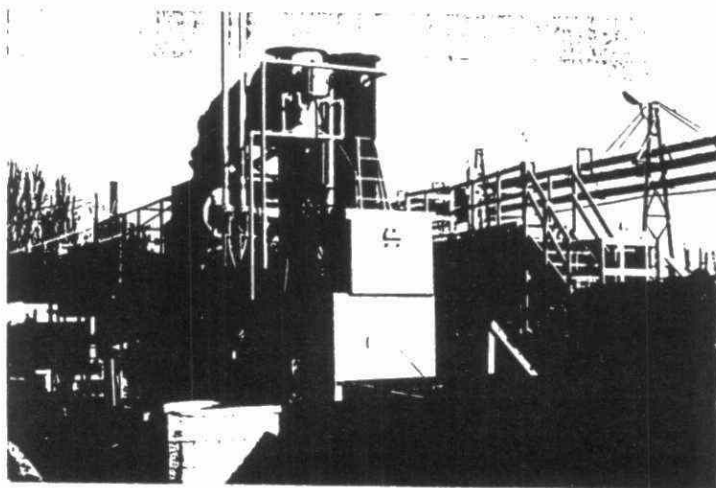
DEMONSTRATION TESTING OF A THERMAL GAS PHASE REDUCTION PROCESS

BY

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1.0 INTRODUCTION

ECO LOGIC has been conducting research on a method of decontaminating hazardous wastes using a patented thermo-chemical reduction process. This process is particularly suitable for wastes that are primarily aqueous, such as harbour sediments, landfill leachates and lagoon sludges. The research to date has been funded by the National Research Council Industrial Research Assistance Program, the Defence Industrial Research Program administered by the Department of National Defence, the Great Lakes Clean Up Fund, the Ontario Ministry of the Environment, the Environmental Technologies Program and ECO LOGIC.

Research and development has focused on bench-scale testing of surrogate compounds, development of a larger lab-scale destructor for testing actual waste samples, and construction of a mobile full-scale field unit for materials and component testing.

2. BACKGROUND

There is a growing sense of awareness and concern about the state of our environment and the lack of appropriate ways of dealing with some of the problems we have created. **ECO LOGIC** was formed in 1986 specifically to address the need for a clean-up tool for one of the most difficult problems, that of severely contaminated aqueous wastes such as harbour sediments, landfill leachates, and lagoon sludges. A technology that could also address stored waste problems such as contaminated soils, contaminated solvents and oils, pesticides, chemical warfare agents, and industrial effluents was considered most desirable. The criteria that **ECO LOGIC** used in developing the process included:

- * destruction efficiency
- * possibility of dioxin or furan formation
- * continuous monitoring and process control suitability
- * suitability for aqueous wastes
- * mobility
- * cost

The patented **ECO LOGIC** process addresses all of these criteria. It is based on the gas-phase thermo-chemical reaction of hydrogen with organic and chlorinated organic compounds at elevated temperatures. At 850°C or higher, hydrogen reacts with organic compounds in a process known as reduction to produce smaller, lighter hydrocarbons. In the case of chlorinated organic compounds, such as polychlorinated biphenyls (PCBs), the products of the reaction are primarily methane and hydrogen chloride. This reaction is enhanced by the presence of water, which can also act as a reducing agent. Bench-scale testing with trichlorobenzene (half of a PCB molecule) has shown that the reduction reaction will achieve 99.9999% destruction efficiency or better. The first measure of a good destruction technology is high destruction efficiency, and the bench-scale testing demonstrated that the **ECO LOGIC** process was capable of this.

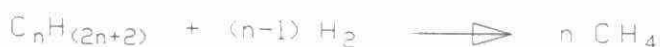
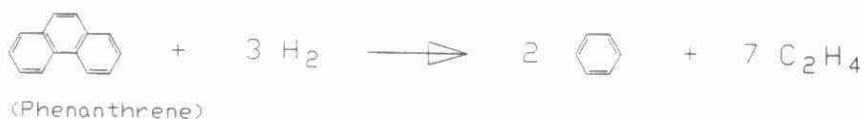
Thermo-Chemical Reduction Reactions

Figure 1 shows the six principal reduction reactions that occur in the ECO LOGIC process. The first is the dechlorination and dismantling of a PCB molecule to produce hydrogen chloride and benzene. The second is the reduction of a polyaromatic hydrocarbon (PAH) compound, phenanthrene, to produce benzene and ethylene. The third reaction is the reduction of a benzene molecule to produce ethylene, and the fourth is the reduction of ethylene to produce methane. The fifth reaction is the reduction of straight-chain hydrocarbons to produce methane. The sixth reaction is known as the water shift reaction and is not a reduction reaction, although it occurs only in a reducing atmosphere. In this reaction, methane and water combine to form carbon monoxide and hydrogen.

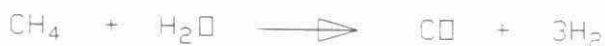
All of these reactions occur simultaneously in the ECO LOGIC process, although to varying degrees and at various rates. The most efficient and fastest reactions are the dechlorination of chlorinated organics and the reduction of multi-ring structures to form benzene. The reduction of benzene to ethylene and the reduction of ethylene to methane occur at roughly the same rate and conversion efficiency, approximately 99%. Straight-chain compounds convert to methane at a higher rate. The result is that about 99% of the organic material input to the process is converted to methane and virtually all the rest is converted as far as ethylene or benzene.

The back reaction from methane in combination with water to form carbon monoxide and hydrogen is much less efficient (20-30%) but, nonetheless, very useful. Since hydrogen is one of the main costs of operation, reactions which generate hydrogen help reduce the overall cost of the process. Unfortunately, this reaction and the conversion of ethylene to methane are both endothermic, so there is an increased energy cost which partially offsets the savings on hydrogen. Another hydrogen-producing reaction is the breakdown of methane to form carbon and hydrogen. This occurs to a limited extent depending on the amount of excess hydrogen in the reducing atmosphere and the reaction temperature.

FIGURE 1
THERMO-CHEMICAL REDUCTION REACTIONS



WATER SHIFT REACTION



The second criterion for process design was elimination of the possibility of dioxin and furan emissions. Because the **ECO LOGIC** process uses reduction reactions, it is not an incineration technology. Incineration processes destroy chlorinated organic wastes by breaking contaminant molecules apart with high temperatures and then combining them with oxygen, usually from air. A PCB waste first fragments to form chlorobenzenes that can combine with oxygen to form dioxins and furans, which are more toxic than the original PCBs. The **ECO LOGIC** process uses hydrogen to produce a reducing atmosphere devoid of free oxygen, and thus eliminates the possibility of dioxin or furan formation.

Other non-chlorinated hazardous organic contaminants, such as PAHs, are also reduced by the **ECO LOGIC** process to smaller, lighter hydrocarbons, primarily methane and ethylene. Because of the tendency of the reaction to produce lighter, more volatile gases, the process lends itself to continuous monitoring of the destruction efficiency. This satisfied the third criterion of process design. **ECO LOGIC** has purchased a very sophisticated on-line mass spectrometer process gas analyser system which is capable of measuring many organic chemicals on a continuous basis. It was used for bench-scale and lab-scale testing, and is now part of the process control system in the pilot-scale demonstration unit. PCB and PAH destruction efficiencies can be measured very quickly by continuously monitoring chlorobenzene and benzene concentrations. The information from the mass spectrometer is sent to the process controller so that an increase in chlorobenzene or benzene concentration (signalling a decrease in PCB or PAH destruction efficiency) halts the input of waste and alerts the operator.

The fourth measure of a destruction technology is its ability to process aqueous wastes. The **ECO LOGIC** process is suitable for many types of waste, including those with a high water content which are very difficult to incinerate. For example, water contaminated with 0.1% (1000 ppm) PCBs can be processed easily with this reaction. The presence of water in the waste actually aids in the destruction process since water itself can act as a reducing agent to help dismantle the contaminant molecules.

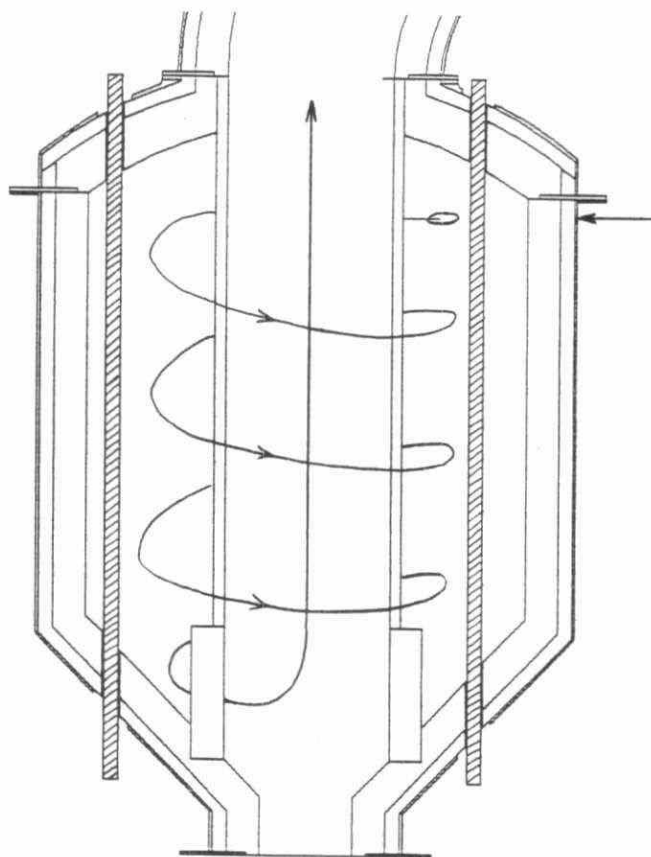
The final criteria ECO LOGIC used in designing the process were mobility and cost. Because the process is not an incinerator, the reactor does not require a large volume for the addition of combustion air. The small reactor size and the capability to recirculate product gases from the reaction make the process equipment small enough to be mobile. As well, the smaller size reduces the capital cost of the process equipment. The main processing costs are for hydrogen, electricity, and personnel. A commercial-scale system processing 100 tonnes per day should be capable of operating for a price of approximately \$500 per tonne of waste processed. This is roughly equivalent to the cost of long-term entombment, with the advantage of actually eliminating the problem.

The research and development required to produce a working pilot-scale model of the process proceeded along four fronts. Bench-scale testing proved the chemical reduction reactions would work with pure compounds with or without the presence of water, and established parameters for residence time, temperature, and appropriate ratios of hydrogen to waste. These tests were conducted at a small scale using laboratory glassware and a quartz tube furnace as a reactor. A larger lab-scale reactor was used to process 5-10 litre quantities of actual waste samples, primarily harbour and lagoon sediment samples. This established the capability of the process to deal with actual wastes in complex matrices. During the bench-scale and lab-scale work, a third thrust of research was the development of a computer model to simulate the operation of a pilot-scale reactor system and to aid in the design of such a system. The fourth phase of research was the construction and proof-of-concept testing of the pilot-scale reactor system. This phase included materials and component testing for coping with a hydrogen- and acid-rich high-temperature reducing atmosphere, and system integrity testing to ensure that the system could achieve temperatures, flowrates and pressures during leak-free operation.

2.2 Pilot-scale Reactor System

Figure 2 shows a schematic of the reactor designed to accommodate the thermochemical reduction reaction. A mixture of preheated waste and hydrogen is injected through nozzles mounted tangentially near the top of the reactor. The mixture swirls around a central ceramic

FIGURE 2
THERMO-CHEMICAL REDUCTION REACTOR

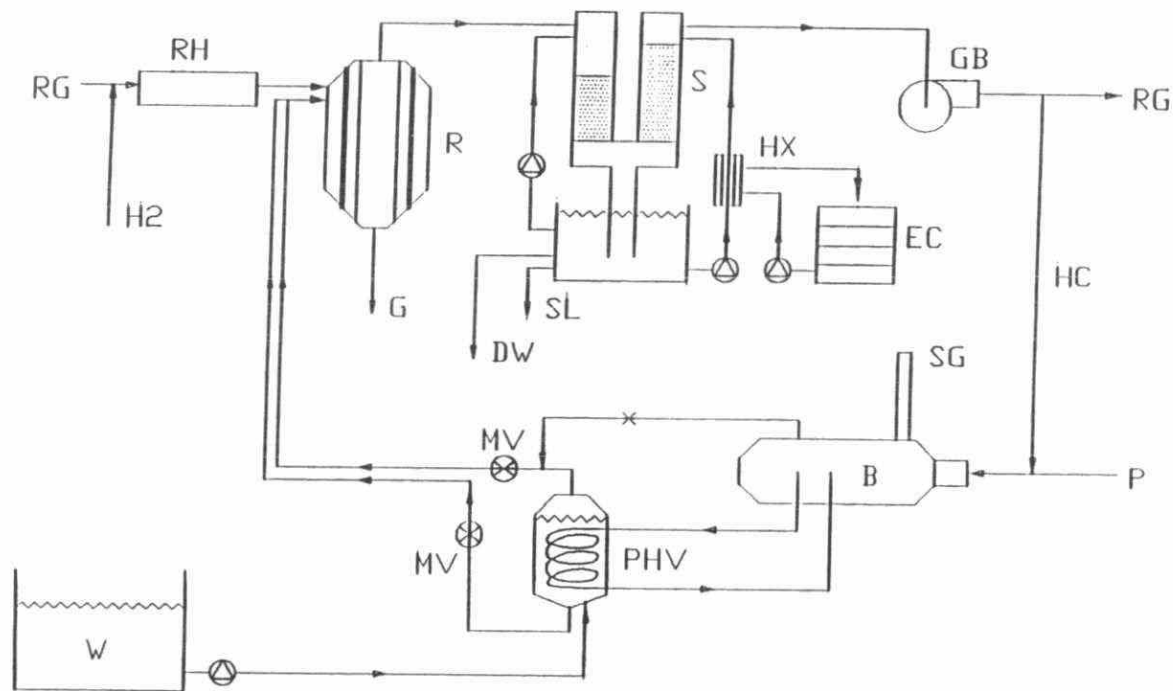


tube past glo-bar heaters and is heated to 850°C by the time it passes through the ports at the bottom of the ceramic tube. Particulate matter up to 5 mm diameter not entrained in the gas stream impacts the hot refractory walls of the reactor, thereby volatilizing any organic matter associated with the particulate. That particulate exits out of the reactor bottom to a quench tank, while finer particulate entrained in the gas stream flows up the ceramic tube into the exit elbow and through the retention zone. The reduction reaction takes place from the bottom of the ceramic tube onwards, and takes less than one second to come to completion.

Figure 3 shows a complete process schematic of the field demonstration unit, which operated at Hamilton harbour. Waste liquid and suspended solids were pumped from a small storage tank (W) to a heat exchanger vessel (PHV) for preheating to 150°C by a small boiler (B). Hot liquid and steam from the watery waste were metered continuously using special metering valves (MV) and injected into the reactor (R) using atomizing nozzles. A mixture of hydrogen (H_2) and recirculation gas (RG) also entered the reactor near the top after passing through a gas-fired heat exchanger (RH). Heavy particulate was removed as grit (G) out the bottom to a quench tank and fine particulate and gases passed up the ceramic tube where the gas-phase reduction reaction took place. Additional residence time was provided by the retention zone elbow and extension pipe prior to the scrubber. Once the gases entered the scrubber (S), they were quenched by direct injection of scrubber water spray. Hydrogen chloride and fine particulate were removed by contact with the scrubber water as the gases passed through the scrubber media. Scrubber water was collected in a tank below via a large water-sealed vent that also acted as an emergency pressure relief duct. The scrubber water was cooled to 35°C using a heat exchanger fed by cooling water from an evaporative cooler (EC). Sludge (SL) and decant water (DW) were the two effluent streams from the scrubber and both were held in tanks for batch analysis prior to disposal.

The gases that left the scrubber consisted primarily of excess hydrogen, reduction products such as methane and ethylene, and a small amount of water vapour. Approximately 95 % of this gas was recirculated back into the reactor after reheating to 500°C, and about 5 % of the hydrocarbon-rich gas (HC) was used as supplementary fuel in the boiler. The boiler used propane (P) as its main fuel to produce steam used in the heat exchanger which preheated the

FIGURE 3
PROCESS SCHEMATIC



waste to 150°C. The only air emissions were from the boiler in the form of stack gas (SG). Since the fuel going into the boiler was very clean and contained no chlorine, emissions from the boiler were insignificant.

The process gas was sampled continuously using an on-line mass spectrometer gas analyser system. A sample was drawn from a location just prior to the scrubber through heated lines to the analyser. In the case of a process upset where total destruction of hazardous organic compounds was not occurring, the on-line mass spectrometer automatically divert all gases into a recirculation mode. Under these conditions, no sidestream gas (HC) would be sent to the boiler and the waste feed would be curtailed. Recirculation would continue until the continuous analysis indicated the reaction was again occurring optimally. During this time, no escape of or incineration of chlorinated organic compounds would occur. During the Hamilton harbour demonstration program, this safety feature was never called upon, but the on-line mass spectrometer proved very useful in evaluating process conditions, changes in feed composition, and destruction efficiency.

The equipment described above was commissioned at Hamilton Harbour on two 45-foot drop-deck trailers. A process control trailer containing the on-line mass spectrometer, process control system, and other analysis equipment was located near the two process trailers. Figure 4 shows a plan view of the pilot-scale reactor system layout as it was set up at Hamilton Harbour. The footprint for the entire process was only 20m by 60m. The equipment was completely self-contained with its own power generator and water supply. Table 2 lists the types of wastes tested at Hamilton Harbour and at bench-scale and lab-scale.

FIGURE 4

PILOT-SCALE REACTOR SYSTEM LAYOUT

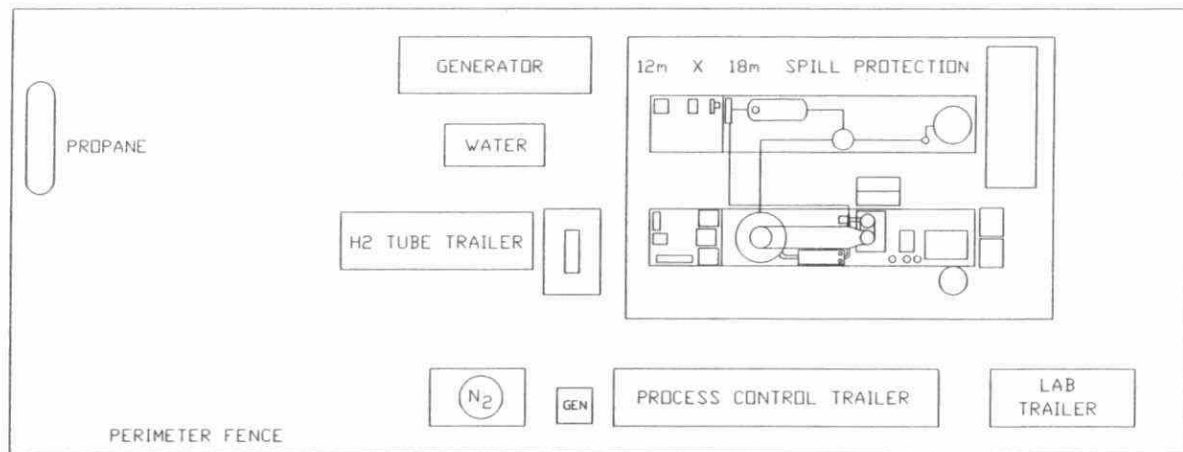


TABLE 1 WASTES TESTED TO DATE

- | | | |
|----|--|---|
| 1) | Pure Compounds | (Bench Scale)
PCB askarel (Aroclor 1254)
Hexachlorobenzene
Trichlorobenze
2,4-D
Methoxychlor |
| 2) | Environmental Wastes | (Laboratory Scale)
(1 Kg/hr) |
| | Hamilton Harbour Sediment | (coal tar, PAH, TCB, Fe, Zn) |
| | Thunder Bay Harbour Sediment | (chlorophenols, wood treatment waste) |
| | Sheboygan Harbour Sediment | (PCBs) |
| | (Audited Program by Environment Canada) | |
| 3) | Full Scale Testing | (2 - 5 kg/min) |
| | Hamilton Harbour Sediment | (3 - 7 T/day coal tar, PAH, Fe, Zn)
(3 T/day as above spiked with 110 ppm PCBs) |
| | (Audited Program by Environment Canada and the Ontario
Ministry of the Environment) | |

PROPOSED DEMONSTRATION FOR FALL 1991

- | | | |
|----|--|----------------|
| 1) | US EPA SITE demonstration | (3 - 5 kg/min) |
| | Composite mixture of liquid PCB, TCE, DCM, and other chlorinated liquids pumped from a municipal landfill. | |

3.0 GENERAL STRATEGY

ECO LOGIC will enter the market to supply hazardous waste destruction services itself with its own machines, and to licence and sell equipment to companies that already supply services, or to licence and sell to large chemical producers or users where ownership is economically advantageous. The service market is the initial focus in order to demonstrate the machine and obtain approvals in jurisdictions where units will be licensed and sold when the technology has buyer (versus user) acceptance.

We would encourage interested parties with organic hazardous waste problems to contact our offices in Rockwood and Ann Arbor. Wastes suitable for destruction using the **Eco Logic** process are listed in Table 2.

This offers potential clients the opportunity to obtain direct information on the application of this technology to the resolution of their hazardous waste problems.

For more information, please do not hesitate to contact:

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TABLE 2 WASTES SUITABLE FOR DESTRUCTION

CHEMICALS:

Non-halogenated / halogenated biphenyls

Non-halogenated / halogenated benzenes

Non-halogenated / halogenated phenols

Non-halogenated / halogenated cycloalkanes

Non-halogenated / halogenated alkanes

Non-halogenated / halogenated dioxins

Non-halogenated / halogenated dibenzofurans

Polyaromatic hydrocarbons

* Note: Halogenated means: Chlorinated
Brominated
Fluorinated

TYPICAL WASTES:

PCBs

Pulp mill wastes

Chlorinated solvent waste

Contaminated coal tars

Solvent still bottoms

Chlorophenols / Wood treatment waste

Pesticide wastes

Landfill leachates

Lagoon bottoms

SEALABLE JOINT SHEET PILE CUTOFF WALLS FOR PREVENTING
AND REMEDIATING GROUNDWATER CONTAMINATION

Robert C. Starr, John A. Cherry, and E. Sam Vales

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INTRODUCTION

The Institute for Groundwater Research (IGR) at the University of Waterloo is developing a new type of steel sheet piling specifically for use as low permeability cutoff walls. These walls are used for controlling the migration of contaminated groundwater and for enhancing the effectiveness of subsurface remedial measures. This new sheet piling differs from conventional sheet piling in that it has joints that can be sealed after the wall has been driven into the ground, and is known as Waterloo Sealable Joint Sheet Piling, or SJSP.

BACKGROUND

Low permeability cutoff walls are commonly used for minimizing the spread of contaminated groundwater at sites such as landfills, waste lagoons, or industrial sites that have on site contaminant sources. Cutoff walls function as low permeability barriers and slow the rate of groundwater advection. The rate of advection through a wall is directly proportional to the permeability and difference in hydraulic head across the wall, and inversely proportional to the thickness of the wall.

There are two wall configurations in profile. Keyed walls extend from ground surface into a low permeability layer, such as clay or silt, that ideally is continuous across the site. Hanging walls do not extend into a low permeability layer at depth. Water can flow beneath a hanging wall, so water levels must be controlled via pumping wells upstream of the wall to prevent advection of contaminated groundwater beneath the wall. However, hanging walls can be used effectively without pumping wells or with very little pumping for preventing migration of liquids that spread near the water table, such as petroleum products, and for preventing migration of soil vapours.

In plan view, the most effective wall configuration is one that completely encircles the zone to be isolated. Non-encircling walls are commonly used, but a network of pumping wells for controlling water levels is necessary for preventing contaminated groundwater from deflecting around the ends, similar to the way that pumping wells are necessary for preventing contaminated groundwater from flowing beneath a hanging wall.

Cutoff walls have long been used in geotechnical applications for minimizing the flow of groundwater into excavations and underflow beneath dams and levees. Cutoff walls are also used for environmental control purposes, typically for either controlling a plume of contaminated groundwater or for isolating a source of groundwater contamination. With a growing awareness of the limitations of pump-and-treat programs for remediating aquifers (Mackay and Cherry, 1989), there is likely to be more emphasis placed on isolating the source of contamination as a control means. Cutoff walls are ideally suited

for source isolation.

Cutoff walls can also be used for enhancing the effectiveness of other site remediation techniques. For example, a keyed cutoff wall enclosure was used for isolating an area where surfactant flushing was used for removing DNAPL from a sandy aquifer (Fountain, 1991). In this application the cutoff wall provides hydraulic control, which minimizes adverse effects due to dilution and prevents migration of fluids away from the remediation area. Cutoff walls can be used for making other remedial techniques feasible. For example, the water table inside an encircling wall can be lowered, and vacuum extraction used for removing volatile organic liquids from the pores.

IGR researchers became interested in cutoff wall construction techniques because there was a need to isolate portions of an aquifer for experimental purposes. We were unable to identify a technique that we were confident would perform satisfactorily, and that could be economically constructed at the relatively small scale required. Compared to the size of a typical cutoff wall project, the walls required by IGR were tiny, and mobilization and setup costs were a substantial portion of the total cost. Walls of a similar size would be very useful for surrounding localized contaminant sources, and for installation in a precautionary mode where spills or leaks of hazardous materials are likely to occur, such as at liquid storage or transfer facilities. Our experience suggests that small installations like these would be relatively expensive if constructed using conventional technology, and hence would not be likely to be built due to the lack of a cost effective technique. However, if a relatively inexpensive, reliable technique for constructing cutoff walls at a small scale was available, areas where spills or leaks are likely to occur could be practically isolated. This would decrease the incidence of groundwater contamination and the liability arising from spills or leaks due to routine operations.

Starr and Cherry (1990) describe a variety of techniques for constructing cutoff walls. In slurry trench methods, a trench is excavated while being kept filled with a suspension of clay and water, which prevents the walls of the trench from collapsing. The trench is either backfilled with a mixture of native soil and bentonite (a soil bentonite wall), or the slurry is amended with Portland cement, which hardens to form a low permeability element (a cement bentonite wall). In grouting techniques, a low permeability material is injected into the ground. Grouting methods include the vibrated beam technique, in which a steel beam is driven into the ground to create a cavity into which grout is injected as the beam is withdrawn, conventional pressure grouting, and jet grouting, in which high velocity jets mix grout with the soil surrounding a borehole to form 'soilcrete' columns or panels. Membrane walls are formed by inserting a low permeability sheet, typically high density polyethylene, into the subsurface.

Conventional steel sheet piling is sometimes used for making cutoff walls for environmental control purposes. However, the use of conventional piling is limited because the open joints between adjacent sheets allow too much water to leak through. Over time, leakage through joints may decline as the joints become clogged as soil is washed in, or as corrosion of the steel blocks the joints with rust. Blockage of joints by these mechanisms is not reliable, so they should not be depended upon for making conventional sheet piling acceptable for environmental control purposes. Obviously, a better situation would be if the joints could be sealed directly.

DEVELOPMENT OF SEALABLE JOINT SHEET PILING

Researchers at the Institute for Groundwater Research have developed steel sheet piling that incorporates joints that can be filled with a sealant after the sheet piles have been driven into the ground (patent pending: E.S. Vales, University of Waterloo). Prototype sealable joint sheet piling was constructed by attaching a steel L section, or an angle iron, to conventional sheet piling. Angle irons were welded to sheet piles to form a cavity adjacent to each joint (Figure 1). The bottom of each angle iron is fashioned in such a way that very little soil enters the joint as the sheets are driven. After the sheets are driven to the required depth, the cavities are flushed to remove any foreign matter and then filled with sealant.

Field trials of cutoff walls constructed with prototype sealable joint sheet piling, which are described in the next section, were successful. These trials indicate that sealable joint sheet piling is feasible from a technical viewpoint. Attaching angle irons to every joint is costly. A less expensive technique for producing a sealable joint would improve the economic feasibility of sealable joint sheet pile cutoff walls. A new joint that provides space for sealant inside the joint itself was designed, which eliminates the need for attaching angle irons at each joint. The modified joint (Figure 2) is formed at the same time that the sheet piling is roll formed, so the additional cost of a sealable joint over a conventional joint is minimal.

The tooling necessary for roll forming a sheet pile section that incorporates the modified joint has been manufactured and installed at a rolling mill. The first production run is scheduled for late October, 1991. The modified sheet piling produced in this first run will be used for field and laboratory performance evaluations, which are scheduled to begin in November.

An important component of this project is selection of sealants that will perform in a variety of subsurface conditions. A joint sealing compound must have low permeability, must bond well to steel after having been emplaced under water, and it must withstand chemical attack by the natural groundwater and any contaminants that are present. It must also withstand freeze-thaw cycles and deformation of the wall due to earth movements. Bentonite based grouts and a proprietary polymer (Dow Chemical) have been used successfully in field tests to date. Additional sealant materials are being evaluated.

FIELD TRIALS

Cutoff walls are generally not constructed in a configuration that allows meaningful field measurement of hydraulic conductivity, so there are few reports of the actual performance of conventional cutoff walls. Estimates of the hydraulic conductivity of cutoff walls are typically based on laboratory measurements using samples of materials used for constructing the wall or samples actually collected from the wall. The as-built performance of a wall depends largely on the presence of imperfections in the wall, which laboratory measurements do not address.

In contrast to the usual situation, several cutoff walls have been constructed by IGR in a configuration that allows meaningful field measurement of hydraulic conductivity. In each case, the test facility is a square or rectangular box, whose walls consist of sealable joint sheet piling. Several test cells have been built at an IGR research site at Canadian Forces Base

Borden. Hydraulic tests have been conducted, and then the cells have been used for other experiments. The cells typically extend through a 3.5 m thick surficial aquifer and 0.5 m into the underlying aquitard, although cells have been constructed to a depth of 11 metres. The aquitard provides a good bottom to the cell, which is necessary for a hydraulic test to be conducted. Another test cell has been built in the clay plains of southwestern Ontario and hydraulically tested. The upper portion of the subsurface at this site is relatively permeable, and the underlying portions are less permeable. The walls of the test cell extend into the low permeability material at depth, which again forms a good bottom to the cell.

To conduct a hydraulic test, the water level inside the cell is displaced from the equilibrium value and the rate at which the water level returns to equilibrium is observed. The rate of water level change depends on the size and shape of the cell, the thickness of the wall, the head difference across the wall, and the permeability or hydraulic conductivity of the wall. The field results are compared with a mathematical model that assumes that water flows uniformly through the entire wall, but not beneath the wall. This yields a single value of hydraulic conductivity for the entire wall, K_{bulk} .

Hydraulic tests of the first cell were conducted before and after the joints were sealed with a bentonite grout. The test conducted before the joints were cleaned or sealed indicates a bulk hydraulic conductivity of 10^{-6} cm/s. Sealing the joints reduced the hydraulic conductivity to 10^{-7} cm/s, or 0.1% of its initial value. This clearly demonstrates the effectiveness of joint sealing in improving the performance of a sheet pile cutoff wall. A larger cell was built using the same style of prototype sealable joint sheet piling and sealed with an improved bentonite grout mixture. The hydraulic test of this cell indicates a hydraulic conductivity of 10^{-8} cm/s.

The test cell in southwestern Ontario was sealed with an organic polymer instead of bentonite grout. The hydraulic test of this cell indicates that the bulk hydraulic conductivity of the wall is between 10^{-9} and 10^{-10} cm/s (Figure 3). There are several differences between this test cell and those at CFB Borden, so it is not valid to conclude that the organic polymer is necessarily a better sealant than bentonite grout. Additional tests at CFB Borden are underway to facilitate this comparison. Nevertheless, the hydraulic tests of the large cell at Borden and the southwestern Ontario cell are quite encouraging, yielding bulk hydraulic conductivity values that are low enough to be acceptable for many environmental control applications.

In jurisdictions lacking regulations that specify a required hydraulic conductivity for cutoff walls, it is common practice to apply US EPA requirements for soil based landfill liners, which require a hydraulic conductivity of 10^{-7} cm/s or less. The State of California requires that cutoff walls at waste disposal sites have a hydraulic conductivity of less than 10^{-6} or 10^{-7} cm/s, depending on the wastes disposed at the site, and a thickness of 0.61 m (California Code of Regulations, 1990). If cutoff wall performance is compared on the basis of the amount of water that flows through a unit area of wall per unit time, i.e. the flux through the wall, then the thickness of the wall and the hydraulic conductivity must be taken into account. Two walls that have the same ratio of hydraulic conductivity to thickness will transmit the same flux through the wall, for the same difference in hydraulic head across the wall. Thus, a SJSP wall 1 cm thick with a hydraulic conductivity of 1.7×10^{-11} cm/s would be equivalent to a wall that meets the more stringent California criteria.

POTENTIAL USES AND ADVANTAGES OF SEALABLE JOINT SHEET PILE CUTOFF WALLS

Waterloo sheet pile cutoff walls have many applications, including:

- shallow containment of spills of lighter-than-water petroleum products, where the wall would be driven to a depth below the water table sufficient to stop the lateral spread of free product near the water table and soil vapours of volatile petroleum constituents;
- deeper groundwater containment of various contaminants such as DNAPLs beneath industrial or commercial properties or hazardous waste disposal sites;
- containment around new or existing commercial or industrial sites or landfills where groundwater contamination is likely to occur;
- temporary containment around sites where soil or groundwater contamination is being remediated.

Cutoff walls can serve as one element in a comprehensive program of site restoration that may involve in situ bioremediation, surfactant or steam flushing or other soil or groundwater treatment processes. At a smaller scale, a portion of a contaminated area can be isolated for pilot scale tests of remediation technologies.

Waterloo sealable joint sheet piling provides several unique advantages over conventional walls:

- rapid installation and sealing;
- minimal disturbance of site landscape during construction;
- health and safety problems and disposal costs that arise when contaminated soil is excavated for slurry trench methods are avoided;
- irregular wall shapes are easily constructed;
- can be constructed in built-up urban areas where equipment access limitations would interfere with construction of other types of walls;
- easily built in areas with high water tables, swamps, or surface water bodies;
- convenient use of chemical-resistant sealants;
- less uncertainty in wall performance;
- positive public perception of wall reliability.

These applications and advantages of sealable joint sheet piling indicate that it can be used for controlling a wide variety of environmental problems.

SUMMARY AND FUTURE WORK

The work conducted to date clearly demonstrates that joint sealing effectively reduces leakage through the joints between adjacent sheets of a sheet pile cutoff wall. Leakage can be reduced to such a low value that sheet pile cutoff walls with sealed joints are suitable for a variety of environmental control purposes. These tests were conducted using a field modified prototype sealable sheet piling. Tooling for manufacturing sealable joint sheet piling that does not require field fabrication has been acquired, and production of the first run of Waterloo sealable joint sheet piling is scheduled for October, 1991. Following production, a field test will be undertaken that involves constructing a test cell that extends to a depth of approximately 15 metres. The cell will be hydraulically tested to evaluate its performance. Identifying and evaluating additional materials that may perform well as joint sealants is underway, and is expected to continue through the winter. If

there are no unforeseen delays, it is expected that Waterloo sealable joint sheet piling will be on the market by the Spring of 1992.

ACKNOWLEDGEMENTS

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ENHANCED OXIDATION TREATMENT OF CONTAMINATED WATER USING HIGH POWERED ULTRAVIOLET LAMPS

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ABSTRACT

The **Rayox**® enhanced oxidation process, employing high powered ultraviolet lamps, is a means for the remediation of contaminated water. There is a growing use of enhanced oxidation technologies to address the need to remove small amounts of waterborne contaminants from the environment. Efforts such as Ontario's MISA program have been implemented to regulate the remediation of contaminated water. The **Rayox**® enhanced oxidation process employs high powered ultraviolet lamps in its technology. This project focuses on further developments of the **Rayox**® technology to allow for building more versatile, powerful, efficient systems and thus to allow for the treatment of a wide variety of contaminated waters. In this paper, results are presented highlighting the benefits of the ultraviolet lamps used in the **Rayox**® technology and the resulting treatment of contaminated waters. Results on the lamp and power supply show that the system operates with comparable or better efficiencies than conventional low pressure mercury lamps, thus producing the added benefits of compact design and reduced capital and operating cost. Quartz fouling, a well known problem for this technology, has been addressed by a major development program resulting in an automated quartz cleaning system. Excellent results have been obtained even in the most problematic process waters. Future development efforts will include further improvements in lamp and power supply design and improved process applications to allow for more efficient and powerful cleanup of contaminated water.

INTRODUCTION

The use of enhanced oxidation processes (EOP) to destroy small amounts of hazardous organic compounds present in contaminated groundwater and industrial process wastewater has become increasingly common in the past few years. EOP treatment offers considerable advantages over traditional oxidation methods employing oxidants such as molecular ozone, hydrogen peroxide or hypochlorite due to the participation of the hydroxyl radical (Hoigne and Bader 1976; Peyton et al 1982). The hydroxyl radical ($\cdot\text{OH}$) is a very powerful oxidizing agent that reacts very rapidly with most organic compounds.

Ultraviolet light is employed in EOP primarily for the generation of hydroxyl radicals from the photolysis of hydrogen peroxide (Sundstrom et al 1986; Mansour et al 1984, Borup and Middlebrooks 1987):



Once generated, the hydroxyl radical reacts with the organic contaminant in one of two ways: it can add to the contaminant, as is the case for olefins or aromatic compounds, or it can abstract a hydrogen atom to form water, as with alkanes or alcohols. Either of these processes initiates a complex cascade of oxidative reactions leading to mineralization.

Ultraviolet light can also initiate the direct photodegradation of pollutants. Compounds that absorb UV light and have reasonable quantum yields for photodegradation are potential candidates. These would include certain aromatic compounds, chlorinated organics and nitrosodialkylamines.

There have been two types of UV lamps used in enhanced oxidation treatments in the past: low pressure mercury lamps and medium pressure mercury lamps. Low pressure lamps have most of their output centered in a narrow band around 254 nm and operate at low powers, typically 40-120 watts. They have good electrical energy to light energy conversion efficiencies (~30%) but their use in EOP is restricted due to their low power and emission in only a narrow wavelength band. Medium pressure lamps have a broad emission in the ultraviolet range with variable output powers up to around 15 kW. These lamps suffer however from low conversion efficiencies, typically around 10%.

Enhanced oxidation treatment using ultraviolet light has not been fully exploited for the solution of real-world problems due to the following factors:

1. Low power output and narrow bandwidth of low pressure Hg lamps
2. Low efficiencies of medium pressure Hg lamps
3. Potential for fouling of quartz sleeves
4. Application of the same configuration of the treatment process without regard to the nature of the contaminant or its concentration.

The third point is of practical consideration, since it is found in continuous operation that materials such as calcium and iron salts deposit on the quartz sleeves of a water treatment system. These deposits effectively block the UV radiation from reaching the water, thus requiring a costly shut down of the system to clean the quartz.

The **Rayox**® enhanced oxidation system has largely overcome the above deficiencies in the use of ultraviolet light for water treatment. This paper describes work carried out on the investigation of lamp/power supply improvements, automatic quartz cleaning and the effect of additives on pilot and full-scale treatment of contaminated groundwater and industrial process water.

EXPERIMENTAL

Experiments were performed in a batch recirculation set-up. The contaminated water was added to a 30 L, 200 L or 300 L holding tank and was pumped through a reactor housing a Solarchem 1 kW, 4 kW, 6 kW, 10 kW or 30 kW lamp. The volume of water treated varied with the lamp size from 20 L to 300 L. The recirculation flow rate was 20-200 L min⁻¹.

The contaminated water was obtained from actual groundwater or industrial process water sites. The water was used as received without pretreatment. The pH was adjusted as necessary with either sulfuric acid or sodium hydroxide. Samples were withdrawn at regular intervals for analysis. The studies of the quartz cleaning device employed an industrial process water, as well as tap water that was spiked with 700 ppm ferrous sulfate, pentahydrate, 10 ppm sodium carbonate and 30 ppm bentonite. The water was recirculated at 50 L min⁻¹ through a **Rayox**® reactor housing a 30 kW lamp.

Samples were analyzed by gas chromatography (GC) or high pressure liquid chromatography (HPLC) as appropriate. GC analysis was carried out on a Varian Model 3300 GC equipped with a flame ionization detector and a 15 m x 0.53 mm i.d. DB-1 column. HPLC analysis were carried out on a Waters Model 6000A pump with variable UV detection and a 15 cm x 0.45 cm i.d. C-18 column.

All reagents were ACS analytical grade and were used without further purification.

RESULTS AND DISCUSSION

Lamp and Power Supply

The basic module of the **Rayox**[®] enhanced oxidation system is comprised of a stainless steel reactor equipped with a quartz sleeve, an automated quartz cleaning device and a Solarchem lamp and power supply. Hydrogen peroxide or ozone is used as the primary source of hydroxyl radicals. ENOX additives, which are proprietary rate enhancing compounds, may be added to improve the treatment of some pollutants.

For a given application, the cost of an ultraviolet light based technology is dependent on the amount of light required to treat the water effectively. This affects both the capital and operating costs by determining the number of lamps needed and the electrical power consumed by the lamps. To minimize the number of lamps required, we have undertaken a development program focussing on all aspects of the lamp system from the power supply to the delivery of photons to the water.

The photodegradation of pollutants in **Rayox**[®] usually follows first-order kinetics so different types of lamps can be compared by determining the slope of the first-order plot of the log of the pollutant concentration versus the kilowatt-hours (kWh) of UV energy consumed. The kWh consumed is proportional to incident integrated light intensity, and hence its use allows for the comparison of lamps without the need to perform chemical actinometric experiments that become very difficult for broad band light sources of large power.

The efficiency of the power supply determines the amount of electricity required to power the lamp. Conventional power supplies for medium pressure lamps generally have operated with efficiencies in the 50% range. This is too low a value for practical use as the operating costs become prohibitive. The proprietary Solarchem power supply has been developed over the past 3-4 years and operates with greater than 90% efficiency. There is thus little wastage of power from the mains to the lamp, which results in lower operating costs.

The amount of electrical input energy converted to UV light is taken to define the efficiency of the lamp. The low efficiency of typical medium pressure lamps (~10%) has made them too expensive for commercial environmental applications. An increase in efficiency would make these lamps more competitive, on an operating cost basis, with low pressure lamps which have efficiencies around 30%. In addition, the wavelength range of the light output is particularly important. It is generally found that most organic pollutants absorb UV light more strongly at shorter wavelengths. Figure 1 presents the UV absorption spectra of four common pollutants and it is readily seen that the UV absorption rises dramatically below 250 nm for all compounds. It is advantageous to have significant output below 300 nm and specifically below 250 nm to improve the photodegradation of pollutants. We have compared 4 lamps for their relative abilities to destroy some model pollutants. The lamps compared were an older model Solarchem 1 kW lamp (Solarchem A), an improved model Solarchem 1 kW lamp (Solarchem B), a Hanovia 2 kW medium pressure lamp and a 25 watt low pressure lamp. Experiments were carried out on two test solutions: 100 ppm 1,4-dioxane with 100 ppm hydrogen peroxide or 10 ppm pentachlorophenol (PCP) with 100 ppm hydrogen peroxide. The results are summarized in Table 1 from which the following general conclusions can be drawn:

- The Solarchem A lamp offers 40 - 60% improvement over the Hanovia medium pressure lamp
- The Solarchem B lamp is 30 - 60% better than the Solarchem A lamp
- The Solarchem B lamp operates at efficiencies similar to a low pressure Hg lamp

Table 1
Observed Rate Constants for Treatment with Different Lamps

$k, \left(\frac{\text{kWh}}{1000 \text{ gal}} \right)^{-1}$ for each lamp				
<u>Conditions</u>	<u>Solarchem A</u>	<u>Solarchem B</u>	<u>Hanovia</u>	<u>Low-Pressure</u>
Dioxane/H ₂ O ₂	0.048	0.078	0.035	0.080
PCP/H ₂ O ₂	0.125	0.165	0.076	0.175

* **Note:** k is the slope of the line obtained from a plot of log c vs. kWh/1000 gallons US.

These findings are of particular practical importance since it would take 1200 low pressure lamps at 25 watts to equal the photons delivered by one Solarchem 30 kW lamp. The relative space and capital cost of the high power lamp system is more favourable. These results demonstrate the improved operating characteristics of the Solarchem lamps that result in lower capital and operating costs for a treatment system. Evaluation of lamp characteristics as well as further lamp developments are on-going projects under this program.

Quartz Cleaning Device

Once photons are generated from a lamp, it is important to ensure they are transmitted to the water consistently. Water containing dissolved metals or solids can deposit a residue on the quartz sleeve during continuous use. We have developed an automated quartz cleaning device to ensure that the quartz remains clean during continuous use. Tests were carried out on two types of water: an industrial process water and a spiked tap water. The results of the tests have shown that the quartz is kept clean for both types of water. The number of cleaning cycles has been varied between 500 and 30,000 with no deposits observed on the quartz. Figure 2 presents a photograph of cleaned versus uncleaned quartz under similar operating conditions and clearly shows the necessity of a cleaning device. This developmental work is now essentially complete.

ENOX Additives

Solarchem has developed five proprietary additives to which it has given the name "ENOX". These additives speed up the destruction of some pollutants. Figure 3 shows the effect of addition of ENOX 510 on the treatment of benzene, toluene and xylene (BTX) in a contaminated groundwater. The addition of ENOX 510 results in a 3 to 4 fold increase in the rate of destruction of the pollutants. The Rayox® process also effectively destroyed the anti-oxidant methyl-t-butyl ether (MTBE). This compound is not amenable to carbon treatment, which has been the standard remediation technique for BTX. Further research is underway on additives for different pollutants.

Treatment Examples

The following examples have been selected to show the type of results that can be obtained with the Rayox® system. The results are from laboratory pilot scale work using 1 kW or 6 kW lamps. Residence times in the corresponding full scale systems range from 6 seconds to 3 min for each 90% destruction of pollutant.

N-Nitrosodimethylamine (NDMA)

NDMA absorbs light strongly at 228 nm and photolyses without the need for hydrogen peroxide. Figure 4 shows results obtained on NDMA treatment using photolysis alone. The full scale system offers four orders of magnitude destruction for only \$0.40/1000 gallons operating cost.

Chlorinated Organics

A groundwater contaminated with a variety of compounds including chlorinated organics has been tested and the results are presented on Table 2. All compounds, including the more refractory dichloroethane, trichloroethane and chloroform, were treated to below discharge requirements.

Table 2
Treatment of Chlorinated Organic Compounds

Compound	Initial Concentration (ppb)	Final Concentration (ppb)	Discharge Requirement (ppb)
Benzene	296	0.75	5
Toluene	0.81	<0.1	24
Chlorobenzene	6.03	0.22	0.3
1,1-Dichloroethane	1100	1	10
1,1,1-Trichloroethane	640	11	200
Chloroform	223	2.87	350
Trichloroethylene	441	0.17	30
Tetrachloroethylene	41.5	0.01	10

Pentachlorophenol (PCP)

A storm runoff water at a wood treating plant is contaminated with up to 5000 ppb of PCP. Figure 5 presents the results of **Rayox**[®] treatment of this water demonstrating the successful results obtained.

SUMMARY

This paper has focussed on ultraviolet light application in enhanced oxidation treatment. Results have been presented showing the affect of lamp type on treatment efficiency. The **Rayox**[®] process has been developed utilizing a high efficiency lamp and power supply, an automated quartz cleaning device and proprietary ENOX additives. These developments result in a powerful system that can treat virtually any organic pollutant to required discharge levels. Further research and development will focus on system improvements in operability and maintainability and process modifications to improve the overall costs of treating contaminated water.

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FIGURE 1

RELATIVE UV ABSORPTION OF COMMON
POLLUTANTS

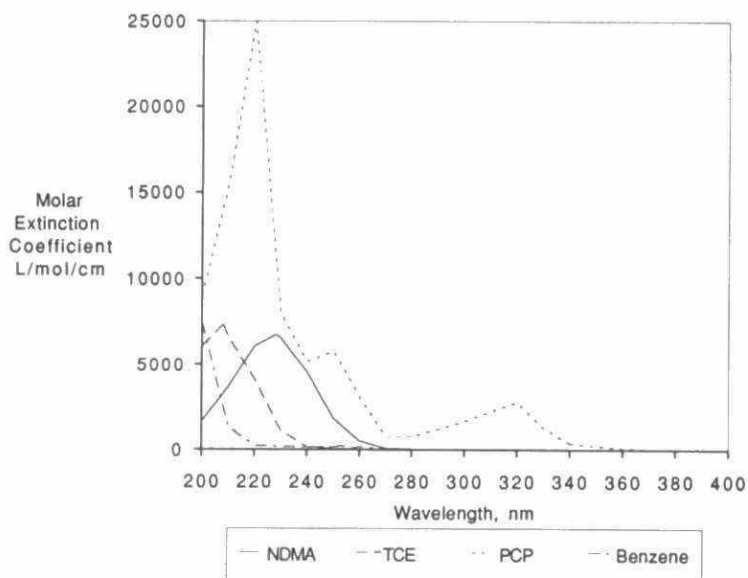




FIGURE 2

THE BENEFIT OF
SOLARCHEM'S
AUTOMATED UV
LAMP CLEANING
MECHANISM

FIGURE 3

RAYOX TREATMENT OF GROUNDWATER
CONTAMINATED WITH BTX

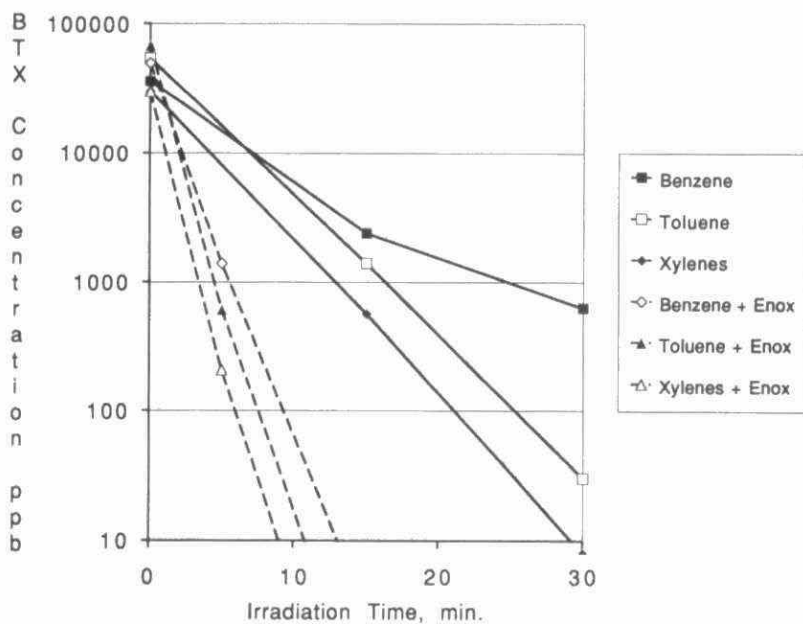


FIGURE 4

RAYOX TREATMENT OF NDMA

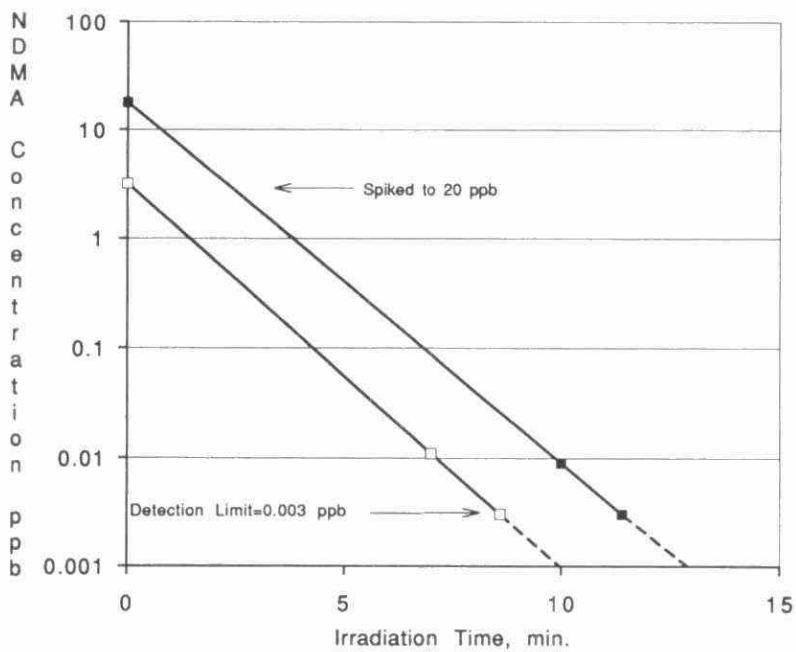
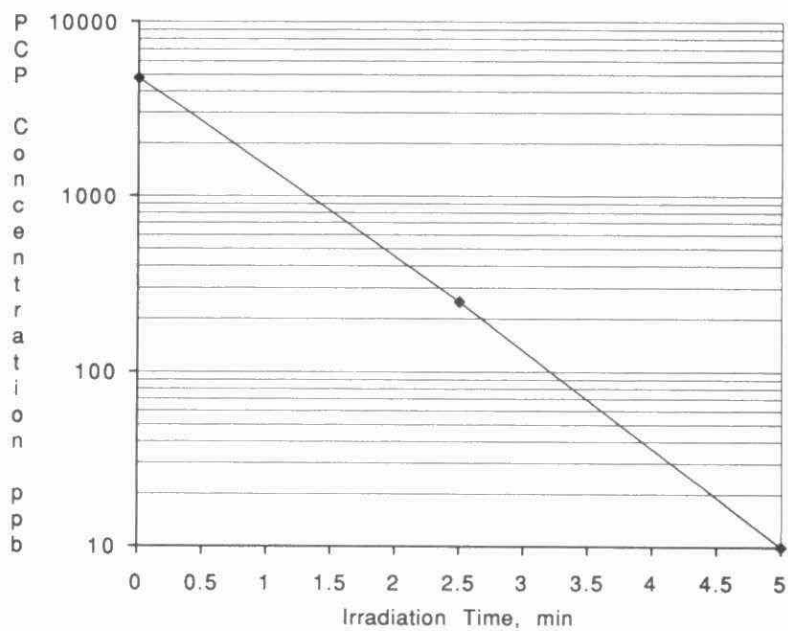


FIGURE 5

RAYOX DESTRUCTION OF PCP
IN STORM RUNOFF WATER



DEINKING OF WASTEPAPER
BY HIGH PRESSURE STEAM TREATMENT FOR PAPER REUSE

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ABSTRACT

A novel steam-explosion technology has been investigated for the effective deinking of selected wastepaper and paperboard furnish for recycling. The features of the process include the continuous pulping of wastepapers at high consistency under high temperature (pressure) steam treatment for short duration, followed by explosive decompression. Deinking of selected wastepaper has been studied both at the laboratory and pilot scales. The steam-exploded pulp has been evaluated for cleanliness (visual and image analysis) and physical and optical properties. Numerous potential advantages in the steam-explosion approach to deinking have been demonstrated from the technical trials. The overall enhanced cleanliness of the deinked pulp could likely reduce the requirement for downstream cleaning after pulping. An additional potential important advantage is the use of furnishes not currently deinkable by conventional technology. Work is currently in progress to expand the range of wastepaper and paper-board furnishes which could most benefit from the new technology.

INTRODUCTION

Wastepaper has recently become an increasingly serious environmental concern due to the current landfill problems in North America. Although limited recycling of selected wastepaper has been implemented in some Canadian cities, the programs are primarily restricted only to newsprint. There are also considerable limitations with the current wastepaper deinking process technologies. Due to the overall ineffective deinking at the pulping stage, the conventional process generally requires the use of chemicals during pulping, as well as multi-stage downstream screening, washing, floating and bleaching treatment to produce a marketable product. A conventional deinking plant therefore suffers from high capital and operating costs. Moreover, it is well known that current processes cannot handle many common low grade wastepaper furnishes and associated

contaminants. It is therefore important that improved wastepaper deinking processes be investigated.

BACKGROUND ON STEAM-EXPLOSION DEINKING PROCESS:

StakeTech has developed and commercialized a proprietary continuous steam-explosion reactor which is currently the only equipment capable of processing wood and agricultural residues at high steam pressure of up to 450 psig on a continuous basis. The company currently owns 120 patents world-wide on its equipment and process technologies for the conversion of lignocellulosic material to cattlefeed, chemical and high yield pulp (S-pulp). Recently the company has extended its process technology for the deinking of wastepaper for recycling.

The steam-explosion deinking process have several unique features. These are distinct from current repulping technologies which generally involves a batch process at low consistency (5-8%), at low temperature (less than 100 C), for long durations (45-60 minutes) and with high energy and chemical consumptions. Steam-explosion processing, on the other hand, is at high steam temperature (and pressure), typically between 160 C to 230 C (up to 400 psig). Pulping is performed at exceedingly high consistency (50% by weight). The process is also continuous, which enables steady-state pulping at all times, thereby ensuring proper process control and uniform product quality. Termination of the pulping reaction is achieved by "explosion", i.e., the sudden release of material from the operating pressure to atmospheric pressure across a discharge valve.

StakeTech, in collaboration with Chesapeake Corp. and Wisconsin Tissue, has carried out steam-explosion deinking of a variety of selected wastepaper and paperboards.

WASTEPAPER RECYCLING:

Deinking of selected wastepapers had been evaluated initially with laboratory scale steam-explosion reactor at the StakeTech laboratory. The steam-exploded pulps, without further treatment, were made into handsheets and evaluated under standard pulp and paper testing conditions, including yields, brightness, physical strength, fiber classification, and cleanliness (visual and image analysis). The effects of various pulping/deinking chemicals, either singly or in combination, on the steam-explosion deinking process were also examined. Studies to date demonstrated superiority of the steam-explosion deinking as

compared to the corresponding laboratory scale conventional deinking with various furnishes. Examples include:

- Coated paper - coated groundwood-free sheets with printing, magazines, brochures, packing plant source
- Office waste - post-consumer colored - file stock, office waste, groundwood-free, sorted
- Groundwood shavings - coated printers coupons, newspaper insert, largely edge trim and low in printings

Numerous advantages were confirmed from these studies. The major ones include:

- reduced pulping time (conventional: 30-60 minutes, as compared to steam-explosion: 1-2 min typically).
- reduced pulping chemicals: data indicated that satisfactory deinking could be achieved with reduced chemicals or even in the complete elimination of pulping chemicals in the steam-explosion process. This is not possible with conventional deinking.
- reduced particle size of contaminants (including ink): the data obtained from image analysis objectively demonstrated a drastic reduction in the size of the dirt particles present in the pulp after the steam-explosion treatment.

Examples:

	Average particle size (sq.mm)	
	Conventional	Steam-explosion
Office Waste:	1.59	0.27
Coated Paper:	0.17	0.06
Groundwood:	0.59	0.21

- enhanced overall cleanliness: this was demonstrated by visual comparisons as well as image analysis (based on the sq. mm. of dirt particles present per sq. ft. of the paper examined). The image analysis is currently considered to be the ultimate test for objectively

evaluating deinking effectiveness.

Example:

	Dirt count (sq. mm/sq. ft.)	
	Conventional	Steam-explosion
coated paper:	295.3	1.9
groundwood:	28.0	5.1

- reduced energy (steam and electricity) consumption in the overall deinking plant (including pulping and downstream cleaning): this was confirmed by comparing a proposed steam-explosion deinking plant with conventional plant (the energy consumption of steam-explosion process is about 70% of those of conventional process). This is a result of the processing at high consistency and for short durations, as well as for reduced downstream cleaning requirement.
- reduced requirement for downstream cleaning, reduced requirement for washing water, and reduced requirement for effluent treatment: these are all logical deductions to the enhanced cleanliness of the resulting pulp obtained from steam-explosion deinking without further treatment.

The laboratory studies were scaled up to pilot testing using StakeTech's commercial size (4 Tonnes per hour) continuous steam-explosion reactor followed by downstream cleaning (if applicable) and paper-making using a pilot paper-machine. The results generally confirmed the scalability of the proposed process and supported the laboratory findings on the potential advantages of the technology.

Overall, results with coated paper suggested that the technology for its recycling might be close to commercialization. Depending on the final paper grades to be determined (based on the market sectors targetted), some of the furnishes tested might require only minor downstream cleaning, such as coarse screening, side-hill screening, and fine screening to become acceptable as a marketable products. The low grade unsorted mixed office waste, on the other hand, might require further technology development. The results on office waste were already demonstrated to be superior to conventional deinking process. With the type of

office waste tested, the current technology could mix in about 15% of office waste with other furnish to manufacture an acceptable tissue product. The results from steam-explosion indicated that it might be possible to use higher levels of office waste (50%) in a mixed furnish. With proper downstream cleaning and again depending on the final end-products intended, it is foreseeable that a 100% recycled office waste may be marketable using the new technology.

Preliminary economic evaluations had been performed on the steam-explosion deinking process for mixed office waste in tissue application. The analysis showed considerable economical advantages of the new process when compared to conventional deinking technologies.

WASTEPAPER BOARD RECYCLING:

Steam-explosion was also demonstrated to be applicable to the recycling of old corrugated containers (OCC) and resin-based wet strength OCC. The high-temperature and short-duration treatment followed by explosive decompression was demonstrated to result in excellent fiber dispersion. The stickies (such as plastics) normally present as contaminants of the OCC appeared to be tolerated well with the new technology. The bulk of the resulting plastics after steam-explosion were shown to be of a size that could be readily removed by coarse screening alone prior to paper making.

The process was successfully scaled up in a pilot operation using StakeTech's commercial scale continuous reactor. Paper produced by a pilot paper-machine in the trial was of an exceptional cleanliness, superior to current commercial products manufactured using conventional technology with extensive downstream cleaning, washing and screening stages. The steam-explosion technology was demonstrated to work well even with heavily contaminated OCC (normally a plant reject). Particularly encouraging results were obtained with wet strength OCC, which currently could not be recycled and was known to cause considerable problems as a contaminant in the OCC recycling operation. The steam-explosion process was able to effect proper fiber dispersion for the recycling of 100% wet strength furnish to be achieved, even in the absence of chemicals and under the short treatment time used.

Attempts were made at the pilot scale to use old newsprint (ONP) as a filler in OCC manufacturing. The low cost of ONP as a result of the ONP glut in U.S. (which was forecasted to last well into the next decades) provided considerable economic incentives for the approach. Evaluations are currently progressing to

determine the extent of strength reduction that may occur at different ONP substitution levels.

With the new technology, the results to date suggested that a simple overall process could be established for the recycling of OCC and wet strength OCC. A simple process scheme might only have to include the following: a shredder, a conveyor (including weightometer and steam or water injection systems to raise the moisture content of the furnish), a StakeTech's Feeder-Digester, dump chest (for furnish dilution), a coarse pressure screen, and a paper machine.

A preliminary economic evaluation of the process had been carried out on the new technology as pertaining to OCC operations. The analysis clearly demonstrated the economic advantages of the new technology when compared with conventional technology.

FUTURE WORK

Overall, the results to date indicated that the technology might be ready for commercialization with some of the furnishes tested. Work under the current project will emphasize on extending the steam-explosion processing technology to the use of wastepaper and paperboard which are not readily recyclable by conventional deinking technologies. The potential effect of the use of chemicals (including surfactants) during steam-explosion for the specific applications will be evaluated.

PLASMA GASIFICATION
FEASIBILITY STUDY
OF
HOSPITAL SOLID WASTE

Presented to
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BACKGROUND

Resorption Canada Limited (RCL) has been actively involved over the past few years in R&D with plasma arc technology, utilizing a specially designed 150KW plasma arc torch in their plasma research facility (PRF) in Gloucester, Ontario. Over the years a wealth of experience and knowledge has been gained in the plasma arc processing of carbonaceous materials, pyroprocessing of ores and concentrates, and in industrial heat applications. A major area of concentration has been gasification, whether for the creation of a usable product gas, for the destruction of a waste material, or for a combination of both. A Plasma Heating System (PHS), when utilized in a reactor vessel with the exclusion of oxygen, can be used very efficiently for the gasification of any carbonaceous material. The resultant pyrolysis provides for virtual complete gasification of all combustibles in the source material, while non combustibles are reduced to a non-hazardous solid residue. Hospital Solid Waste (HSW), with its high carbonaceous content, is such an application, which combines energy production with the destruction of a waste material with minimal environmental pollution. Plasma gasification of HSW is, therefore, a very substantial alternative to current forms of disposal.

The energy and environmental characteristics of the plasma gasification of carbonaceous waste materials were studied over the past four to five years when RCL completed extensive experimentation with Municipal Solid Waste (MSW) through cost sharing agreements with the Ontario Ministries of Energy and Environment, Ontario Hydro and other third party firms; Electric Power Research Institute (EPRI), Arthur Gordon Environmental Evaluators Limited, RSP Environnement Inc. and Centerior Energy.

PROJECT SCOPE AND STATUS

With this experience in plasma processing RCL submitted an unsolicited proposal to the Ontario Ministry of the Environment (MOE) for cost sharing consideration to investigate the plasma gasification of HSW. This proposal was approved for an October 1991 commencement with an expected completion of mid 1992. The project will establish optimum operating parameters and then determine the energy and environmental characteristics at these operating parameters. Facility modifications, as identified through the MSW experimentation, will be effected to further improve the environmental results. The PRF is currently being updated for the commencement of first testing.

THE EXPERIMENTAL PROCEDURE

The RCL PRF is shown in Figure 1. Each experimental run covers a duration of up to eight hours, depending on the requirements of the individual run. HSW is very similar in composition to MSW; therefore, the feeder developed for feeding MSW will also be used for the HSW. The pathological and infectious components of the HSW will be simulated, on the recommendation and approval of the MOE, which will negate the requirement to design and build a special feeder, and permit more accurate ultimate and proximate analyses for subsequent comparison purposes. Steam, or water, can be introduced at varying rates, as required, to provide additional oxygen to react with the free carbon forming additional combustible gaseous products.

System control is very versatile and enables one operator to process varying amounts of material up to the maximum capability of the facility depending on the type of material being processed. The operator has continuous readout of critical operational parameters of the process.

The process temperature, as monitored at the inside wall of the reactor vessel, is approximately 1100 deg C. Once this operating temperature is achieved, HSW is weighed, fed into the vessel and processing commences. The optimum feed rate for the process is determined through a combination of the temperature inside the vessel and the amount of product gas being generated.

Solid residue is permitted to accumulate in the bottom of the reactor vessel and is removed at the end of the experimental run by hydraulically tipping the entire vessel. The solid residue is poured into a bed of silica sand.

The volume of product gas generated is continuously recorded on a Bailey gas meter, and a log of all torch operating parameters is maintained to facilitate full analyses at a later time. The product gas chemical composition is monitored with a Gas Chromatograph to ensure a good cross sectional reading of the product gas quality. The product gas can be combusted in a burner directly from the gasification process, or it can be flared on exit.

Environmental sampling and analyses are independently conducted on the product gas, the products of combustion of the product gas and the solid residue.

PLASMA GASIFICATION CHARACTERISTICS

Energy and environmental characteristics of the plasma gasification process were determined through the previous experimentation with MSW.

The energy characteristics as determined within the RCL PRF and as extrapolated through known process improvements to a 50 to 75 tonne per day commercial size installation are

as follows:

	<u>LABORATORY RESULTS</u>	<u>COMMERCIAL SIZE EXTRAPOLATION</u>
Conversion Ratio (Energy Out/In)	1.65:1	4.30:1
Overall Efficiency	56.0%	72.2%
Product Gas/Refuse HV Ratio	0.847	0.868
Product Gas HHV (BTU/SCF)	282.15	282.15 (max)
Dry Refuse/Slag Weight Reduction	5.37:1	5.37:1
Wet Refuse/Slag Weight Reduction	8.93:1	8.93:1
Refuse/Slag Volume Reduction	184:1	184:1
El Energy per Tonne Refuse (KWhrs)	1595	612

Commercial size extrapolation includes known improvements obtainable through the use of a larger size and more efficient plasma arc torch, the use of additional refractory lining in the wall of the reactor vessel and economies of scale for heat losses. Conversion ratio is defined as the energy available in the product gas compared to the electrical energy input to the process which created the product gas. A conversion ratio of 4.3:1, therefore, means that for every BTU of electrical energy input to the process through the plasma arc torch there are 4.3 BTUs of usable energy in the product gas. This ratio does not consider any energy recovery from the sensible heat in the hot product gas, the hot solid residue or the torch cooling water, which account for the majority of the 28% losses.

The heat balance for the process is shown in Figure 2, and the chemical composition of the product gas and the solid residue are shown in Table 1. Representative air emission data for Polychlorinated Dibenzop-dioxins (PCDDs), Polychlorinated Dibenzofurans (PCDFs), Chlorophenols (CPs), Chlorobenzenes (CBs), Polychlorinated Biphenyls (PCBs) and

Polychlorinated Aromatic Hydrocarbons (PAHs) are shown in Table 2. Leachate toxicity levels for the solid residue are shown in Table 3.

Where possible, the environmental characteristics tables include comparable statistics from National Incinerator Testing and Evaluation Program (NITEP) reports for the Quebec City (Que) and the Prince Edward Island (PEI) incinerators, along with Ontario regulation 309 and Quebec regulation limits, and the Canadian Council of Ministers of the Environment (CCME) guidelines. The Que and PEI incinerators do not have extensive pollution control equipment; Que has an Electrostatic Precipitator and PEI is a dual stage incinerator. However, these installations were selected by NITEP as having designs which exemplified projected future trends in incinerator technology in Canada and they underwent an extensive optimization/modernization upgrade to a state-of-the-art design immediately prior to testing. In marked contrast, the RCL PRF was not designed with pollution control in mind, rather its design and optimization concentrated only on the gasification process.

The environmental results are self evident, indicating that almost all aspects of the plasma gasification process are superior to the most optimized incineration processes. PAHs are the only emission found to be higher than incineration levels; however, in retrospect this would be expected since PAHs are destroyed in an oxygen rich environment, whereas the plasma gasification process is designed to minimize the amount of oxygen present. In the plasma gasification process PAHs will be destroyed primarily through an efficient combustion of the product gas.

The solid residue from the process is monolithic with toxic element leachability levels, even on fully crushed samples, substantially lower than the Environmental Protection toxicity standards currently in either the Ontario or Quebec regulations. These levels are also significantly lower than the normal levels of toxicity found in the ashes of conventional incineration processes. The results shown in Table 3 are from fully crushed samples, whereas, if these samples had been prepared and analysed in accordance with accepted procedures for a monolithic material, even lower results would have been obtained.

PLASMA GASIFICATION ADVANTAGES/INNOVATIVE FEATURES

The plasma gasification process appears to have substantial cost, size, operability and environmental advantages/innovative features over current disposal methods. These features include:

HEAT SOURCE

- a. Independent heat source - provides flexibility since it can be controlled at will and can process material from very little to its maximum capability and still operate at an optimal operating point. This relative ease of controllability makes it particularly amenable to off-peak processing to substantially reduce input electricity charges;
- b. Higher processing temperature and heat transfer rate constantly available - conducive to the breakdown of most chemical bonds with the prospects of forming less complex and non-polluting compounds; and
- c. Processing temperatures can be varied and maintained - controllable at the will of the operator to meet

varying input material composition demands.

PROCESS

- a. Very little oxygen (much less than for stoichiometric combustion) present through the process - results in minimal combustion and a richer product gas;
- b. Unaffected by variations in moisture content in input material - optimal operation can be maintained;
- c. Two distinct processes before exhausting to the atmosphere - distinct separation between the gasification process and the combustion process to minimize problems associated with the direct combustion of the input material;
- d. Reaction of the moisture in the input MSW with the free carbon to form additional combustible products - results in the virtual complete removal of carbon from the input material;
- e. Effluents/residues can be recirculated - catch material from pollution control equipments, as may be required through different installations, can be reinput into the process to eliminate separate disposal requirements; and
- f. No new smokestack required - combustion can be accommodated in existing boilers to negate the requirement for an additional smokestack with its inherent high degree of public rejection.

PRODUCT GAS

- a. Flammable gas produced as a discrete product - provides

system flexibility. It caters to varying energy demand needs since the product gas can be used immediately, piped elsewhere or stored for later use; and

- b. Small volume to be treated by scrubbers - cost and complexity of pollution control equipment is minimized.

PHYSICAL

- a. Small size - results in lower capital costs; and
- b. Installation versatility - small size enhances integration into existing facility superstructure, and also installation largely underground for noise abatement and to present a more aesthetically pleasing appearance.

SOLID RESIDUE

- a. Non-combustibles in the input material, such as glass, metals, dirt, etc., melt and chemically combine into a dense, inert and non-hazardous solid;
- b. Immense volume reduction from shredded refuse to slag - would be even greater with as-received;
- c. All input objects completely melt and are unrecognizable in the output solid residue;
- d. Molten slag acts as a secondary heat transfer medium on the bottom of the vessel;
- e. Inert, non-leachable solid residue may have cost effective commercial uses - indications are for use in construction, insulation and packaging industries; and

- f. No continued use of landfill - frees up valuable real estate and alleviates continuing liability from leachate toxicity.

DISADVANTAGE

The primary disadvantage of the plasma gassification process is the operating costs associated with the high electrical consumption. This cost is somewhat offset by the higher volume of product gas generated and, in some cases, it can be further alleviated by operation at lower power during peak hydro usage periods. For example, demand charges can be 30%-90% lower, depending on the hydro district, for operation between 8PM and 8AM and all day Saturday and Sunday, which potentially can be used to advantage. Cogeneration could also be very advantageous to offset the hydro operating costs. Initial assessment of cogeneration indicates that the full process could be virtually self sustaining.

CONCLUSION

Historically, environmental standards are invariably made more stringent and improved standards are developed whenever new developments show that they can reliably meet more stringent limits. Plasma gasification shows the potential of being able to set such new standards.

ENCLOSURES

FIGURE 1 - RCL PLASMA RESEARCH FACILITY

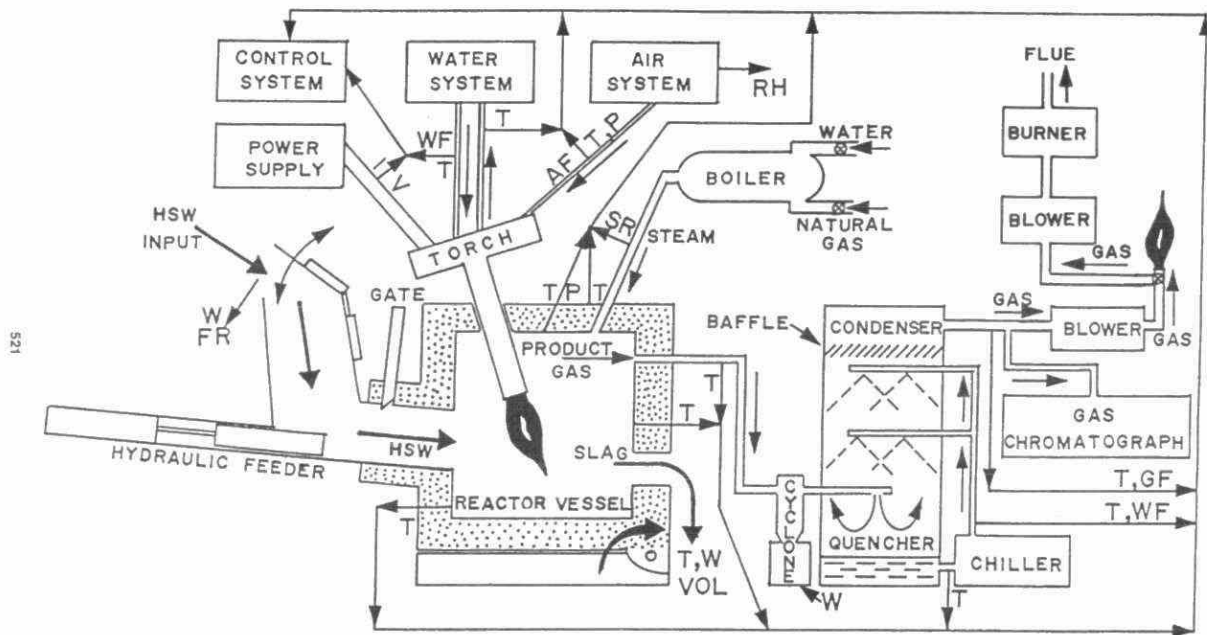
FIGURE 2 - MSW COMMERCIAL SYSTEM HEAT BALANCE

TABLE 1 - PRODUCT GAS AND SOLID RESIDUE CHEMICAL COMPOSITION

TABLE 2 - AIR EMISSIONS - PLASMA GASIFICATION OF MSW

TABLE 3 - LEACHATE TOXICITY - PLASMA GASIFICATION OF MSW

FIGURE 1 - PLASMA RESEARCH FACILITY



I—CURRENT (AMPS) V—VOLTAGE (VOLTS) T—TEMPERATURE (DEGREES C) GF—GAS FLOW (SCFM)

P—PRESSURE (PSIG) W—WEIGHT (LBS) RH—RELATIVE HUMIDITY (%) WF—WATER FLOW (GPM)

FR—FEED RATE (LBS/HR) SR—STEAM RATE (LBS/HR) AH—AIR FLOW (CFM) VOL—VOLUME (CF)

RESORPTION CANADA LIMITED

MUNICIPAL SOLID WASTE YIELDS

FIGURE 2: COMMERCIAL SYSTEM HEAT BALANCE
(FROM R&D RESULTS FEBRUARY 1988)

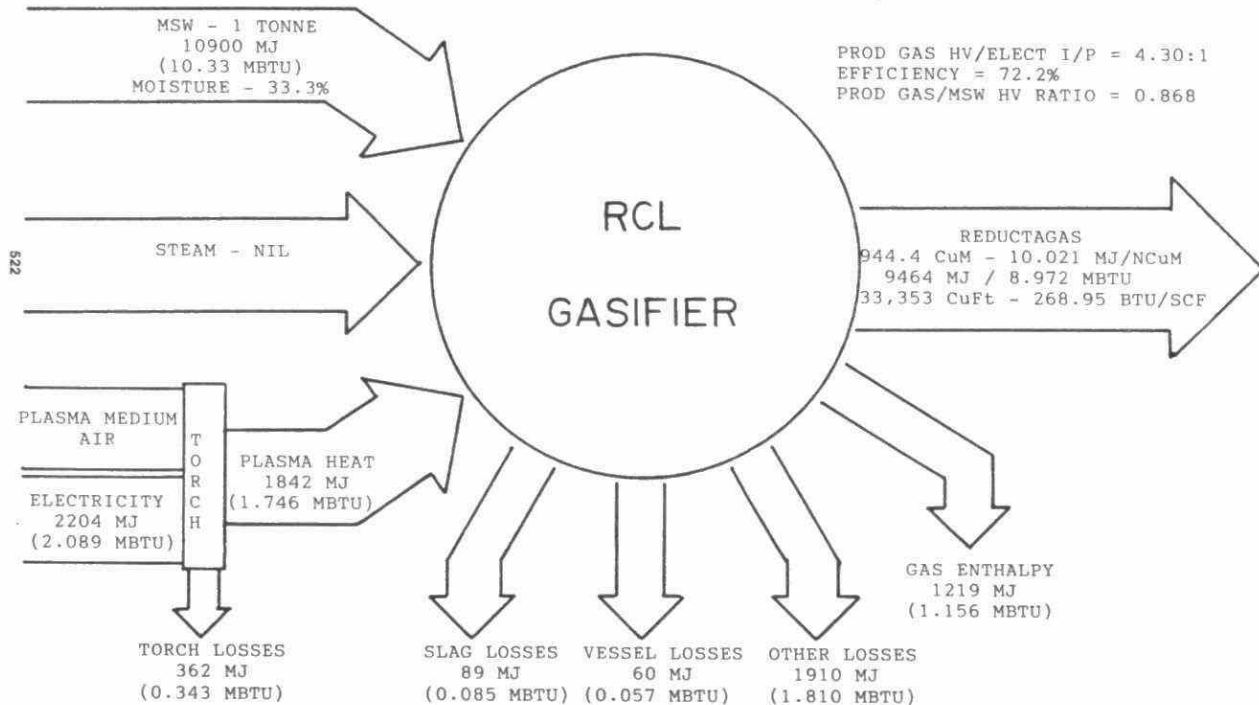


TABLE 1: PRODUCT GAS & SOLID RESIDUE CHEMICAL COMPOSITIONPRODUCT GAS COMPOSITION - (at max heating value)

HYDROGEN	41.2%
CARBON MONOXIDE	29.7%
NITROGEN	17.0%
CARBON DIOXIDE	8.3%
METHANE	3.2%
OXYGEN	0.3%
ACETELYNE	0.2%
ETHYLENE	0.1%

SOLID RESIDUE COMPOSITION - (major elements only)

<u>ELEMENT</u>	<u>ELEMENT PERCENT</u>	<u>OXIDE PERCENT</u>
ALUMINUM	9.5	18.0
CALCIUM	10.4	14.6
IRON	2.3	3.3
MANGANESE	0.1	0.2
PHOSPHORUS	0.4	0.8
POTASSIUM	1.6	1.9
SILICON	23.2	49.7
SODIUM	3.8	5.1
TITANIUM	0.6	1.1
TOTAL	51.9	94.7

TABLE 2: AIR EMISSIONS - PLASMA GASIFICATION OF MSW
(CONCENTRATIONS PER NORMAL CUBIC METER)

<u>COMPOUND</u>	<u>RCL LAB RESULTS</u>	<u>EXISTING INCINERATOR(1)</u>	<u>CCME GUIDELINES</u>
<u>SEMI-VOLATILE ORGANICS</u>			
<u>TOXIC EQUIVALENCY</u>			
DIOXINS (NANOGRAMS (NG))	0		
FURANS (NG)	0 - 0.5		
TOTAL	0 - 0.5		0.5
<u>ACTUAL VALUES</u>			
DIOXINS (NG)	ND	19 - 298(A)	
FURANS (NG)	3 - 10	44 - 306	
CHLOROPHENYLS (MICROGRAMS (UG))	ND	5 - 24	
CHLOROBENZENES (UG)	0.6 - 3	3 - 10	
POLYCHLORINATED BIPHENYLS (UG)	ND	2 - 7	
POLYAROMATIC HYDROCARBONS (UG)	1900 - 15000	4 - 22	

(1)(A) - EXISTING INCINERATOR AT QUEBEC CITY - NITEP REPORT EPS 3/UP/5

TABLE 2: AIR EMISSIONS (CONTINUED)
(CONCENTRATIONS PER NORMAL CUBIC METER)

<u>COMPOUND</u>	<u>RCL LAB RESULTS</u>	<u>EXISTING INCINERATOR(1)</u>	<u>CCME GUIDELINES</u>
<u>METALS</u>			
ANTIMONY (MILLIGRAMS (MG))	0.02 - 0.05	0.5 - 2.6(B)	
CADMIUM (MG)	0.004 - 0.03	0.6 - 0.9	
CHROMIUM (MG)	0.02 - 0.08	0.03 - 0.1	
LEAD (MG)	0.2 - 0.6	8.4 - 15	
MERCURY (MG)	ND	0.5 - 0.9	
NICKEL (MG)	0.02 - 0.08	0.2 - 0.5	
<u>ACID GASES</u>			
HYDROGEN CHLORIDE (MG)	0.1 - 0.6		75
HYDROGEN FLUORIDE (MG)	0.07 - 0.7		
HYDROGEN BROMIDE (MG)	ND		
NITROGEN OXIDE (PPM)	158 - 305	169 - 246(A)	
SULPHUR DIOXIDE (PPM)	66 - 69	128 - 225	
<u>PARTICULATES</u>			
MG PER NORMAL CUBIC METER	2.4 - 9.9	167 - 247(B)	20

(1)(A) - EXISTING INCINERATOR AT QUEBEC CITY - NITEP REPORT EPS 3/UP/5

(B) - EXISTING INCINERATOR AT PRINCE EDWARD ISLAND - NITEP REPORT EPS 3/UP/1

Table 3: LEACHATE TOXICITY - PLASMA GASIFICATION OF MSW
(MILLIGRAMS PER LITRE - CRUSHED SAMPLE)

<u>ELEMENT</u>	<u>RCL LAB RESULTS</u>	<u>ONTARIO REGULATION</u>	<u>QUEBEC REGULATION</u>
ALUMINUM	0.03 - 0.18		
ANTIMONY	ND - 0.006		
ARSENIC	ND	5.0	
BARIUM	0.03 - 0.1	100.0	
BERYLLIUM	ND		
BISMUTH	ND		
BORON	ND	500.0	
CADMIUM	ND	0.5	0.1
CALCIUM	1.15 - 3.3		
CHROMIUM	ND	5.0	0.5
COBALT	ND		
COPPER	0.07 - 0.09		1.0
IRON	0.57 - 1.79		17.0
LEAD	0.01 - 0.02	5.0	0.1
LITHIUM	ND		
MAGNESIUM	0.15 - 0.2		
MANGANESE	0.01 - 0.02		
MERCURY	ND	0.1	0.001
MOLYBDENUM	ND		
NICKEL	0.005 - 0.01		1.0
PHOSPHORUS	ND		
POTASSIUM	0.05 - 0.1		
SELENIUM	ND	1.0	
SILICA	0.68 - 1.16		
SILVER	ND	5.0	
SODIUM	0.1 - 1.3		
STRONTIUM	ND		
TELLURIUM	ND		
TIN	ND		
TITANIUM	ND		
VANADIUM	ND - 0.015		
ZINC	0.02 - 0.05		1.0
	ND - NOT DETECTED		

VOLUME II

SESSION E

ENVIRONMENTAL TECHNOLOGIES PROGRAM

POSTER PRESENTATIONS



DEVELOPMENT OF MEMBRANE TECHNOLOGY FOR DRINKING WATER PRODUCTION: TREATMENT OF COLOURED WATER

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Some drinking water supplies, particularly those in the northern part of Ontario, are highly coloured. Although this may not represent a direct threat to the health of consumers, these substances contribute to the formation of trihalomethanes when the water supply is chlorinated. Conventional water treatment technologies using coagulation and sedimentation followed by filtration do not effectively remove these compounds, and concern over potential health effects has resulted in increasingly stringent government limitations for trihalomethane content in treated waters.

Membrane technology offers one promising solution to this problem. Although membranes are not yet commonly used in municipal water treatment, ultrafiltration and nanofiltration are emerging technologies which offer significant potential as effective processes for removal of particles and higher molecular weight organic compounds. Nanofiltration has recently been successfully applied for treatment of coloured groundwater supplies in Florida, and application for coloured surface supplies is currently of interest. Intensive research efforts are being directed at developing membrane processes to a commercial stage for application in potable water treatment.

This study was undertaken to determine the technical and economic feasibility of membrane nanofiltration technology for the removal of colour and trihalomethane precursors from drinking water sources. In-house testing of the performance of several commercially available membranes in parallel with near commercial membranes developed by Zenon will be performed on three different water supplies. Two membranes will then be selected for on-site pilot testing at one of the test sites.

In-house baseline tests using a synthetically prepared water source with colour levels up to 200 APHA have recently been completed, and testing on the first natural water supply is underway. The first test site is a small community in northern Ontario drawing from a surface supply where colour levels range from 40 to 150 APHA.

Pilot testing is expected to take place in early 1992, and results will be used to develop a proposed plant design. The process will be evaluated against conventional treatment and a complete technical and economic assessment will be carried out. The potential for commercialization and market estimates will be also be assessed.

Expert System Software Development for Assessment of Solid Wastes Leaching and Disposal - 'LANDIS' Expert System

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Abstract

LANDIS (LANd DISposal) is an expert system based software decision tool. Its purpose is to guide the user through a solid waste assessment process to determine the suitability of disposing a specific waste in a specific landfill site. Expert system rules control the evaluation process and incorporate the expertise necessary to render final conclusions and recommendations from the evidence gathered during a LANDIS consultation.

The LANDIS program consists of several interactive modules under the control of the expert system rules and knowledge base:

- relational database management system for storage, retrieval and analysis of waste physical/chemical characteristics
- HELP landfill design and hydrological model with a separately-designed graphical control interface (Hydrological Evaluation of Landfill Performance, ver. 2.05, USAE Waterways Experiment Station, Vicksburg, MS; widely-used and validated with full scale sites)
- location/climate database management system for maintaining and adding climate data to the HELP model (eg. Canadian sites)
- consultation user-interface system

The primary types of information requested and generated during a consultation are:

- waste physical and chemical characteristics,
- waste leaching characteristics,
- target landfill design, local climatic factors and landfill hydrology,
- controlling leaching mechanism,
- concentration and mass flux of contaminant(s) leaving the landfill projected over time (inorganic species only can be determined at this time).

LANDIS has been designed from the outset to be applicable to regulatory solid waste assessments. Current regulatory practice utilizes the results of physical/chemical characterization and one or more bench-scale leaching protocols (protocol types are specific to each provincial jurisdiction) to determine whether a solid waste is suitable for land disposal. LANDIS provides a logical extension to this practice by *incorporating the characteristics of the target landfill site* while utilizing the standard procedures and protocols to determine waste characteristics. In addition, LANDIS utilizes a battery of leaching protocol types in conjunction with waste

properties and target landfill hydrology to determine the controlling leaching mechanism and apply the leaching protocol test procedures most suited to this leaching mechanism. With this information, the concentration and mass flux of the contaminants of interest leaving the landfill over time (source terms) can be calculated and projected in the future. The suitability of disposal of a specific waste in a specific landfill is determined, therefore, by the concentration or mass loading of specific contaminant species projected over time. The methods used are a combination of standard laboratory testing protocols and the fully-validated HELP landfill hydrological model.

LANDIS is also a very useful tool for conducting 'what if' hypothetical scenarios. This capability can be employed for purposes of design or to screen waste and/or landfill disposal options (eg. waste solidification/stabilization options, effect of capping landfill, liner properties, etc.). Waste characterization/leaching data, either from individual waste entries or as generic waste type properties, can be utilized in a screening consultation run. Consultations can be interrupted and re-started at the same point at a later time. Thus, multiple consultations can be run simultaneously.

LANDIS is normally operated as an integrated multi-module application. However, the waste characteristics/leaching database and HELP model (with graphical interface) can also be run as independent programs. The database system is, in itself, a valuable resource which can be used to study and analyze leaching data using built-in statistical tables and a query-based report generator. The original HELP model character-based interface has been upgraded to a 'Windows-like' graphical interface which greatly enhances its usability while preserving its computational core.

The full integrated program will run on a IBM PC-AT compatible computer equipped with a VGA graphics card/monitor, math co-processor and hard disk (386/486 CPU recommended). A fully functional prototype has been developed along with technical documentation, a user guide, and tutorial. LANDIS is intended for use by regulatory agencies or other users who have an understanding of solid waste leaching and familiarity with laboratory bench-scale leaching protocols. The program has not been designed for a novice user.

This project is jointly funded by the Environmental Technologies Program of Environment Ontario, the Canadian Electrical Association and Dearborn Chemical Co. Ltd. Over the next two years the program will be expanded to include a wider variety of waste types and more information specific to Canadian climate and cold weather effects, such as frost penetration, to be included in the HELP model. System validation will then be conducted based on a review of the knowledge base by domain experts and one or more full-scale site waste disposal assessments. A commercial version of the program and documentation will then be released.

CONVERTING SEWAGE SLUDGE INTO LIQUID HYDROCARBON

THE "OFS PROCESS"

D. Martinoli, SNC Inc., Oakville, Ontario

In 1982, Environment Canada, through its Wastewater Technology Centre in Burlington, Ontario, undertook to verify the conversion of wastewater treatment sludge into liquid hydrocarbon.

The principle of low temperature gasification of sewage sludge to produce fuels has been known for several years. France issued a patent to Saburo Shibata entitled "Procédé de fabrication d'une huile combustible à partir de boue digérée" in November 1938. However, in 1982, two German scientists from the University of Tübingen in Germany demonstrated the mechanism by which the sludge is converted into oil.

They heated dried sludge to 350°C in an oxygen-free environment for approximately 30 minutes producing oil, char, gas and water. Oil yields ranged from 18% to 27%, and char yields from 50% to 60%. The oil had a calorific value of approximately 39 MJ/kg, and the char, approximately 15 MJ/kg.

Environment Canada preliminary bench-scale experiments validated the previous results. A large technology demonstration and development program led to the design and construction of two 1 t/day pilot plants. The first one was built in 1986 in Hamilton, Ontario, and the second one in 1988 in Perth, Australia. These two pilot-scale plants have a capacity of 40kg/h each, which is equivalent to 1 t/day of dry sludge.

The bench-scale reactor has been used to evaluate process performance and to obtain data on a number of sludges. This reactor, which is 5 cm in diameter and 100 cm long is made up of two zones, the first for volatilization and the second for conversion. WTC has operated the system using over 25 different sludges from Canada, the United States, Australia and the United Kingdom.

Based on the results of the bench-scale reactor tests, Environment Canada contracted with a subsidiary of SNC in 1985 to design, construct and operate the first pilot plant to confirm the bench-scale results and the projected energy savings. This 1 t/day pilot plant, includes a 1 m³ sludge storage bin, a sludge feed system, a 25 cm diameter by 3.5 m long reactor, a char discharge system, a direct-contact condenser, an oil-water separator, and a propane burner to heat the reactor. It is similar to the bench-scale reactor described above, except for the method of heating the reactor.

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In September 1986, this unit was delivered to the Woodward Avenue Sewage Treatment Plant in the Hamilton-Wentworth Regional Municipality and has since been operated with three different sludges, namely the raw sludge from Atlanta, and digested sludges from Rockford and Hamilton.

A second generation pilot-scale reactor was built in Perth, Australia in 1988.

Originally, this unit was located at the Subiaco Wastewater Treatment Plant of the Water Authority of Western Australia (WAWA) in Perth in Western Australia. This unit was moved to Sydney and was used to test the sludge from the Malabar Wastewater Treatment Plant.

The object of the testing program was to collect all the process data necessary to design a full-scale demonstration plant. The tests were also used to evaluate any environmental impacts. The results of the test program have been analyzed and found well within the requirements of existing regulations.

The tests were carried out on both raw and digested wastewater sludge. As expected, the oil yields from raw sludge are generally greater than from digested sludge, because in the sludge digestion process, volatile materials are destroyed and not converted into oil. The oil yields go from a high of 46% for raw sludge to a low of 13% for digested sludge, and are a function of the reactor's operating temperature and the source of the sludge. Oil viscosity is lower for digested sludge than for raw sludge. It has also been observed that viscosity decreases with increasing temperature, but no definite relationship has yet been identified between operating temperature and the oil's calorific value.

Fine tuning continues in the Canadian and Australian pilot plants, and some modifications have been made to improve operation and performance. The results from these two plants, which have been operated with different sludges, have generally confirmed that:

- * The system is capable of converting the sludge and producing usable car.
- * The heat distribution on the reactor shell supplies a conversion temperature which allows the relatively low solids retention times.
- * Throughput capacity of 40kg/hour can be reached.
- * The oil, reaction water and NCG can be separated.
- * The performance is similar to that of the bench-scale reactor.

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Conceptually, an OFS plant includes, in addition to the OFS reactor, 3 processes that have been used and proven in sludge management systems:

- * Dewatering, to produce a sludge cake of 30% or more dry solids.
- * Drying, to bring the sludge to 95% or more dry solids.
- * Energy generation, to convert the char, the non-condensable gases (NCG) and the reaction water into thermal energy.

The OFS process has the following advantages:

- * Energy considerations - The OFS system is expected to be an extremely energy efficient option. This is due to several factors such as thermal drying and the conversion into oil by a chemical process with an efficiency greater than 90%, rather than by a biological or thermal process. Consequently, this system is a net energy producer and approximately 50% of the sludge's gross energy is recovered in the form of hydrocarbon. Equally important is that it is the only system to produce a liquid hydrocarbon, easy to store and transport, and essential for several industries in the petroleum sector as special additives.
- * Design and operating flexibility - The OFS process requires less space, thus a smaller building and a reduction in the capital cost combined with a reduction in the operating costs such as heating, electrical maintenance, etc. Because an OFS plant includes a number of independent sludge processing units, plant expansions and retrofits are much easier and less costly.
- * Environmental superiority - The OFS system provides complete pathogen destruction, odor control, metal immobilization and air pollution reduction.
 - Viruses and bacteria are completely destroyed in the three consecutive thermal processes, namely drying (30-90°), conversion (450°C for more than 30 minutes) and combustion in the fluid-bed furnace (900°C).
 - Odor control in an OFS plant is facilitated by the fact that each step of the process is totally enclosed and that the air from the process is used as combustion air for the fluid-bed furnace.

- The fate of the heavy metals in the OFS process has been well defined. The numerous laboratory and pilot plant data indicate that the heavy metals, with the exception of mercury, are found in the char of the system. In the combustion of the char, the heavy metals are retained in the ash and are converted into non-leachable oxides and silicates.
- A significant feature of the OFS process, as compared to incineration, is that the fluid-bed furnace is fed by dry fuels rather than by wet sludge. The quantity of exhaust gas generated by the fluid bed, and the need for air pollution control equipment are thus greatly reduced. The fluid-bed furnace is in fact known for its very high environmental and energy efficiency. In addition, its hot flue gases are used to dry the sludge.

* Cost effectiveness - With the revenues from hydrocarbon sales, and the fine tuning of the system which will lead to a decrease in construction costs, we can expect a short-term objective of \$250/ton for dry sludge treated with the OFS process.

Enersludge inc., a Canadian company 50% - owned by SNC inc. of Montreal, and 50% - owned by Campbell Environmental limited (CEL) of Perth, Australia, signed an exclusive license agreement with Canadian Patents and Development limited. Following this agreement, SNC began marketing and carrying out OFS projects in North America, while CEL covered Australia and the southeast Asian countries (Pacific Rim).

Currently, three projects are at various stages of development:

1. **The Municipality of Metropolitan Toronto**, a 48 tpd capacity plant for Highland Creek Treatment Plant in Scarborough, Ontario.
2. **The Halifax Harbour Cleanup inc.**, a 20 tpd capacity plant for the combined Halifax-Dartmouth wastewater treatment complex which is under design.
3. **The Sydney Wastewater Board**, for the Malabar Treatment Plant in Sydney, Australia, to eliminate ocean dumping.

USE OF CHEMILUMINESCENCE TECHNIQUES IN PORTABLE, LIGHTWEIGHT, HIGHLY SENSITIVE INSTRUMENTS FOR MEASURING PAN, NO₂, NOx, AND O₃. *J.W. Drummond, L.A. Topham, G.I. Mackay, and H.I. Schiff. Unisearch Associates Inc. 222 Snidercroft Road, Concord, Ontario Canada L4K 1B5

Small battery powered instruments for the sensitive detection of nitrogen dioxide (NO₂), and ozone (O₃) will be described. Because of their portability, they are ideal for studies of indoor air quality, measurements from aircraft, and other mobile platforms where power may be limited. For example, a miniature balloon borne sonde, based on the chemiluminescence between luminol and NO₂ has recently demonstrated the ability to measure ambient mixing ratios of NO₂ between ground level and an altitude of 33 km. A converter to enable measurements of NOx by the Luminox^R NO₂ analyzer is presented. The model LOZ-4 ozone monitor will be described. Ozone is measured by using its chemiluminescence with the dye Eosin Y. In comparison with ethylene based ozone detectors, the LOZ-4 has better sensitivity, and does not require a troublesome and flammable gas. The O₃ measurement is virtually interference free. In contrast, the widely used photometric O₃ measurement technique has demonstrated significant interferences in polluted air (presumably due to organic compounds that absorb light at the 253.7 nm wavelength). Finally, a sensitive instrument for measuring PAN will be described. It uses a gas chromatograph to separate the PAN from the whole air sample before detection by Luminox^R techniques. Field measurements for all species are presented.

ENVIRONMENTALLY RATIONAL SOLUTIONS: GOOD BUSINESS DECISIONS WITH POSITIVE ENVIRONMENTALLY IMPACT. A.A. Wakeford, Administrator, ProActive Recycling Inc., Owen Sound, Ont. N4K 3R2 (519-371-6511)

A team of three experienced men; an ink technician, a printer and a businessman from Owen Sound, a beautiful tourist area decided to leave a legacy of cleaner land and water to the next generation through the development of ink recycling for large printers. By mortgaging their residences and with the support of a 3 R's grant they have designed, tested, modified and placed in production a mobile ink recycling system.

Year I consists of recycling a single type of printer's ink; coldset black. Emphasis was placed on meeting 'virgin' specifications of Tack, Grit, Grind, Viscosity and Shade. Testing was done and after one year a successful unit was created. Commercialization has paralleled this creation, fighting at all times the perception that 'recycled' is 'inferior'. The sleeping giants, the ink companies, do take some exception to this intrusion into their territory.

Year II introduced the expansion into recycling of all four colours of heatset inks. Currently we are expanding into other ink types, such as sheet fed waterbase and forms ink. Paralleling our technical expansion is our commercial expansion into Quebec and the United States.

Our objective remains a cleaner environment without sacrificing industry's bottom line. Both objectives are possible with rational environmental decisions to recycle and reuse our urban resources.

VOLUME II

SESSION F

PREVENTION, TREATMENT AND REMEDIATION

VERBAL PRESENTATIONS

FROM WASTE TO SECONDARY RESOURCE

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ABSTRACT

The lack of quality control is the first problem we usually encounter in our efforts in the re-cycle or re-use of waste materials generated by manufacturing industry. The variations in properties of wastes are often due to lack of attention in the process in which they are produced and due to contaminations inside the plant.

As secondary resource, it must meet certain requirements including consistency so that its user would have an efficient operation.

The requirements in the current market of a secondary resource must be brought to the attention of waste generator for potential modification of the manufacturing operation to re-classify the waste as a product with due respect. Communications concerning feasible changes in manufacturing progress on one hand and possible financial rewards in the market of secondary resources on the other are essential in converting wastes to secondary resources. As an example, the situation in the re-use of slags from metals industry will be discussed.

1. INTRODUCTION

In order to facilitate our discussion the definition of waste and secondary resources must be clarified. Let us start with an example of a primary resource. In Northern Ontario we have deposits of taconite, a mineral containing about 30% iron, of several billion tonnes. In the 1940's these rocks were of no particular economic value. Due to the development of new processes in mineral dressing, in the 1950's we had several billion tonnes of taconite iron ore, three mines were in operation from the 1960's to 1980's. The great increase on land transportation caused all three Ontario mines to close in the late 1980's, therefore, several billion tonnes of taconite has changed from iron ore to rock again. It is clear that our available technology and market place determines the economic value of particular resources.

The waste in this presentation is industrial waste which is produced in a manufacturing process. It has a negative price at the plant gate of its generator.

A secondary resource is a by-product of a manufacturing process. It has a positive price at the plant gate of its generator.

It must be clearly stated that changes in the market place and technology may convert waste to secondary resource as well as from secondary resource to waste.

2. CHARACTERISTICS OF WASTES

The most important characteristic of waste is that it appears to be useless in the present form. The second important feature is that you and/or your government want to have it removed from your property.

It is no fun and down right unpleasant to examine one's own garbage, we don't know and don't want to know our garbage any better. We just want the garbage men to take these bags away, period! We can imagine that executives in companies do not like their industrial waste any more than we do our domestic equivalent. The first problem we encounter on the way to overcoming the "uselessness" of waste, is the lack of knowledge regarding the properties of waste. In our homes, we put all kinds of wastes in one bag (before the Blue Box Program). In industry, there is no reason, at least in the past, not to put many kinds of wastes together to result in a very large variation of properties. To initiate the equivalent of the Blue Box Program in large manufacturing companies, certainly is the first step in solving the waste problem.

When we try to market our waste as secondary resource, bearing in mind that we must be very specific about the quality of our product.

3. QUALIFICATIONS OF A SECONDARY RESOURCE

In a modern day society and competitive market, no business could survive without an efficient operation and a good product. The company which uses the secondary resource

(which was a waste) to substitute for part of their usual raw materials, is no exception. A secondary resource must meet the specifications including consistency as demanded by the user, otherwise, it will become industrial waste soon after its rejection from the market.

4. THE STEEL INDUSTRY AND DOWN STREAM CONTRACTORS

As an example, the description of waste materials in the steel industry is greatly simplified for our discussion. In ironmaking and steelmaking operations, waste oxides produced may be classified into the following three categories:

- (a) Waste: steelmaking dust
- (b) Secondary Resource: pelletized blast furnace slag
- (c) Waste/Secondary Resource: steelmaking slag and ironmaking dust

These waste oxides are usually handled and processed for their disposal or as secondary resources by contractors in the steel plants. Ironmakers and steelmakers are very busy ensuring that their production, the cost and the quality of iron and steel are on targets, has no practice or need up to the present time, to look into their garbage cans. And they don't!

5. FROM WASTE TO SECONDARY RESOURCE

The case of steelmaking dust will be discussed as an example. At steelmaking temperatures of 1600°C, volatile heavy metals which are originated in the recycled steel scrap, vaporize and subsequently are oxidized and collected as dust

for air quality control. The major components of dust are iron oxides, zinc oxide, lead oxide and calcium oxide.

The contents of zinc and lead oxides, ranging from 1% to 30% Wt., is a measure of the severity of the problem at hand as well as the potential of converting the dust to secondary resource. At landfill sites, zinc and lead may be leached by rainwater, leading to further contamination of the environment. On the other hand, if the zinc and lead content in dust is high enough and technology is efficient enough to extract these metals from the dust instead of ore, dust becomes a secondary resource.

Many new processes for treating steelmaking dust to produce iron and zinc-lead alloys or zinc and lead oxides have reached pilot plant stage and a very few at the commercial scale of operation. However, their economic viability is yet to be established. The conversion of steelmaking dust to secondary resource will accelerate once either the cost of treating dust goes down due to a technological break-through, or the cost of its disposal goes up due to a shortage of landfill space or other reasons.

6. FROM SECONDARY RESOURCE TO WASTE

In the Hamilton area, the annual production of steelmaking slag is about 800,000 tonnes. Most of it is being used as aggregate for asphaltic concrete for road pavement. The contractors handle slag from the liquid state to crushing, sizing and finally marketing to the construction industry.

Steelmaking slag is chemically unstable and can only be used as aggregate in asphaltic concrete. It has excellent properties for road surface, except it may expand due to hydration resulting in cracking of the pavement. For some reason, this problem becomes more pronounced recently. If this problem can not be solved soon, we will witness the conversion of 800,000 tonnes of secondary resource to waste.

7. EFFORTS SHOULD BE MADE COLLECTIVELY

In our efforts to convert waste to secondary resource or upgrade the secondary resource to a higher priced product (as in the case of ironmaking slag), we are talking of two types of activities:

- (i) Technical development of new products based on given or slightly modified raw materials (i.e., wastes). It may involve physical and chemical processing.
- (ii) Marketing new products (secondary resources) to old or new customers.

Future success, in our opinion, depends largely on our collective efforts to overcome the following two difficulties:

- (i) The lack of technical capability in this problematic area to define the nature of the problem.
- (ii) The lack of communication through the chain of companies from the generator of waste to the processor to the user of secondary resources.

INDUSTRIAL DEVELOPMENT

Our industrial development plan begins in Metro. Our technology is sufficiently advanced that we are now working to arrange financing for our first plant. We intend to purchase the St. Lawrence Starch ethanol distillery in Port Credit, which recently shut down. It is an excellent fermentation, distillation and dehydration plant for our intended products. We plan a 6 month trial period in place, and then to dismantle the distillery and move it to another site. The total cost of the demonstration run, the dismantling and re-erection of the distillery, real estate, civil works, etc. is estimated to be \$12 million. We project building several such distilleries at a cost of about \$20 million each, for a total of more than \$100 million, so this is a rather substantial project. If we get into MSW separation, it becomes still larger.

The St. Lawrence distillery has a capacity of 20 million liters of ethanol per year, and would convert waste paper at the rate of 70,000 tonnes per year. We are also meeting with the Paperboard Packaging Environmental Council, and hope they will want a dedicated distillery with paperboard packaging (Rice Krispies boxes, etc.) as feedstock. At this point we have no deep concerns about the supply of feedstock, or the markets for the fuel ethanol. In Ontario, the government has a 13 cents per liter subsidy for ethanol in motor fuel, which is enough to be attractive to distributors and blenders. At the 10% ethanol level, which the regulations permit, ethanol-gasoline blends are a premium grade, and command the same price as premium fuel. This practice is very widespread south of the border. The only problem we anticipate is being able to assure customers of an adequate supply of ethanol in the early stages, so it is a race to get enough ethanol into production: a chicken and egg situation, but one that has been solved elsewhere.

The wholesale value of the product of the first distillery, 20 million liters at 30 cents per liter, has an income of only \$6 million. To be adequately profitable, a tipping fee of \$60 per tonne would have to be charged. This is much lower than current tipping fees. At the same time as we reduce the volume of MSW to landfill by half, we convert the garbage disposal burden to a handsome profit.

When we have 5 such distilleries based on waste papers, the accumulated surplus profits should generate enough funds to build a garbage separation plant. Such separation plants are expensive. We would take the light fraction and produce ethanol from it, at a good profit. Other

Iogen, in Ottawa, which has contributed to research on synthesis of cellulase enzymes and is now studying synthesis of cellobiase, but has not yet begun to study waste paper, as far as we are aware. In the U.S., the Solar Energy Research Institute (SERI) in Golden Colorado, has also studied enzymatic saccharification, but has not, as far as we know, proceeded to pilot plant enzymatic studies, nor to the study of waste paper.

There are also several groups studying the major alternative process, acid hydrolysis of biomass. In the U.S. the Tennessee Valley Authority (TVA), in Muscle Shoals, Alabama, has a pilot plant which is now studying municipal solid waste by acid hydrolysis. In Canada, Professor Chorner, of the Universite de Sherbrooke, Quebec, and St. Lawrence Reactors of Toronto, have the largest such pilot plants. TVA, in anticipation of severe corrosion problems with the sulphuric acid they use for hydrolysis of biomass, has built their equipment of zirconium, a very expensive, and perhaps unnecessary, decision. St. Lawrence Reactors has taken a different approach. They employ hydrochloric acid as the hydrolysis catalyst, and the tubes in their continuous plug flow reactors are made of a Swedish steel which, after more than 5 years of operation, shows no signs of corrosion. The results so far published on the acid hydrolysis process show yields far below those obtained with the enzyme process.

In Europe, a consortium of German and Italian companies has built an interesting pilot plant which follows a partial acid hydrolysis with enzymatic saccharification. This is a very new plant, for which no data are as yet available. In Japan a consortium of companies has built a very elaborate pilot plant which includes every process step that anyone could think of, in the south of Honshu near Miyazaki. The data from it are not impressive. There are many research groups studying bioconversion of biomass to ethanol in Japan, funded by the New Energy and Industrial Technology Development Organization (NEDO), by brewers and distillers, by the Research Association for Petroleum Alternatives Development (RAPAD), and the Fuel Alcohol Research Association (FARA). Also they have very large enzyme production factories, one of which supplied most of the enzyme we used in our early work. The resources available to Japanese scientists involved in biomass research are fabulous. I have been privileged to visit a few of their installations, and am sometimes depressed at the contrast between their facilities and staffing, and ours here. Yet Canada can be proud of the contributions of our scientists to this important area of science and technology, not just in relative terms, but absolutely. In many respects, we are well ahead.

BEHAVIORAL ECOLOGY OF THE EASTERN SUBTERRANEAN
TERMITE IN ONTARIO AS A BASIS FOR CONTROL

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and

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ABSTRACT

Subterranean termites are serious structural pests which cause economic losses on the same order of magnitude as fire. Their range in Ontario has steadily increased over the last sixty years despite municipal efforts and a Ministry of Environment-funded grant program to control and contain them. Conventional chemical control is environmentally unacceptable and fails to kill the colony which simply expands its foraging range following chemical treatment. The objectives of our research have been to develop a control strategy to kill whole termite colonies based on sound fundamental knowledge of behaviour and ecology, to demonstrate the ability to extirpate localized populations, and thus to provide a means for municipal-wide termite eradication in Ontario. Through mark-release-recapture studies we have gained a much clearer understanding of the population size, biomass, density, dispersion, territory size and foraging dynamics of northern populations of this species. Populations number in the millions, covering thousands of square meters, with typical densities of about 2,000 per square meter. The caste system, population structure, and seasonal development have been studied. To understand over-wintering, experiments have been conducted on metabolic heat output and termite movement in relation to soil temperature. To understand summer moisture stress, studies were conducted on water imbibition, water transport, and regurgitation on soil and wood for microhabitat humidity control. The effect of soil structure on water availability, rates of tunnelling, and shelter tube construction were investigated. Above-ground foraging on trees is a strong behavioral tendency of *R. flavipes* which is not exhibited by other *Reticulitermes* species in North America. Tree foraging by *R. flavipes* has been further investigated. These biological findings have enabled us to improve aggregation trap designs and trap efficiencies--as many as 42,000 termites have been taken from a single trap and over a half million termites taken from a single plot. A Trap-Treat-Release approach has been investigated in which the trapped termites are treated in ways which induce delayed mortality and transmit slow-acting toxicants or vector microbial biocontrol agents back into the colony. Several borate compounds have been studied as slow-acting transmissible stomach poisons. Some show considerable promise and can be applied either in baits or as cuticular dusts. We have shown that cuticular loading of dusts can be greatly increased with various nontoxic spray paints used as sticking agents. Using sprays to increase loads of borate dust we have found effective lethal ratios of treated to untreated as low as 1:20. Several fungi, viruses, and nematodes have promise as biocontrol agents by the Trap-Treat-Release approach. Physical treatments could be used in conjunction with pathogens. Physical treatments such as gamma and X-ray, and UV irradiation all induce delayed mortality. Inter-colony agonism has not been discovered in Ontario suggesting a possible founder effect and reduced genetic variability which raises the possibility of using treated termites from one colony to invade and vector pathogens or slow-acting toxicants into another colony. Further study will improve trapping and treatment protocols and should make the Trap-Treat-Release approach a viable tool for eradicating termite populations from Canadian cities.

Introduction

The eastern subterranean termite, *Reticulitermes flavipes*, is now known to infest 32 municipalities in southern Ontario. The human population of Ontario is 9,426,100 of which 3,783,161 (40%) reside in the 32 infested municipalities. This termite species was first recorded from Point Pelee in 1929 and its introduction to Toronto is believed to have occurred between 1935 and 1938 (Kirby, 1967). As of 1988, the Toronto Housing Department reported that 5,730 houses had been treated, or about 5% of the total housing stock of the city. These infestations occur on 437 blocks or about 15% of the total city blocks. The average cost for chemical treatment and wood-soil separation in 1988 was \$3,272. From 1975 to 1989 the Ontario Ministry of the Environment expended \$5.6 million on its Termite Control Program which assisted home owners in covering the cost of treatment. Unfortunately, the spread of termites has not been curtailed and the assistance program has now been suspended. With financial backing from municipal, provincial and federal sources the Urban Entomology Program in the Faculty of Forestry at the University of Toronto was established in 1987 explicitly to conduct applied research for the development of an integrated pest management approach to control the eastern subterranean termite. Dr. J. Kenneth Grace directed the program from 1987 to 1990. Dr. Tim Myles has directed the program since 1990.

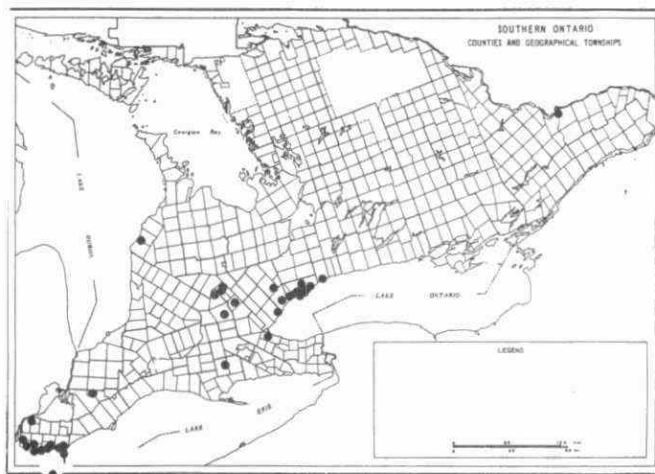


Figure 1. Known distribution of termites in Ontario. Infested municipalities include: Windsor, Malden Centre, Colchester, Kingsville, Leamington, Oxley, Harrow, Mersea, Gosfield South, Amherstberg, Point Pelee, Pelee Island, Dresden, Kincardine, Guelph, Elora, Fergus, Nichol, Woolwich, Kitchener, Hamilton, Oakville, Brampton, Mississauga, Toronto, North York, York, Etobicoke, Scarborough, East York, Pickering, Markham, Old Windham(?).

Population Dynamics and Foraging Behaviour

Esenther (1970) was the first to use the mark-release-recapture method to estimate *R. flavipes* population size in the field. His population estimates varied from 0.32 to 9.5 million termites. Grace et al (1989) and Grace (1990) used similar methods and arrived at estimates of 0.72 to 3.2 million termites. An intensive mark-release-recapture study on a single plot in Kincardine was conducted during the summer of 1990. The daily rate of mortality was adjusted to minimize variation among sequential 4-day estimates. A final population estimate of 4.2 million termites was calculated. The mark-release recapture method is also useful for measuring the foraging territories of colonies. Grace et al reported territories up to 1,091 m². The Kincardine study in 1990 indicated a foraging territory of 1,344 m². Typical lot sizes on the east side of Toronto are 25 X 100' indicating that a single colony could overlap five or more entire properties.

Penultimate nymphs overwinter and transform to final stage nymphs and then to winged alates from March through May. Winged alates do develop in Ontario populations but flight records are uncommon due to the inconspicuous nature of most flights. The most abundant reproductive caste in field populations are the nymphoid supplementary neotenics and field evidence indicates a seasonal peak of neotenic development also occurs in the spring. Eggs, small larvae and presoldiers are found through the summer months.

Local populations are undoubtedly more stressed by winter coldness than by summer dryness but in both cases are able to cope well enough in urban-suburban environments. Our experiments have shown that termites do not generate appreciable metabolic heat in aggregations and overwintering therefore depends on finding subterranean hot spots associated with buried wood. Dead trees, stumps, sewer systems and heated structures are likely to provide overwintering hot spots. In the laboratory we typically operate growth chambers at temperatures from 85-90°F at which termites do well and have the highest rates of food consumption. However, the soil temperature preference experiments we have found that when given a choice termites will actually seek much cooler soil temperatures (50-70°F).

Experiments on termite movement relative to soil structure have indicated significant effects of particle size on rates of soil penetration, soil tunnelling, and shelter tube construction. Termites are unable to lift particles heavier than 7 mg (about twice the weight of a large worker) and rarely move particles weighing more than 4 mg. Generally termites are unable to move particles with diameters greater than 1.4 mm and rarely move particles greater than 1 mm in diameter. When soil particles exceed 2.8 mm in diameter the interstices between the particles are just large enough for small worker termites to crawl through. Thus, a mixture of sand composed of particles with diameters ranging from 1.4 to 2.8 mm is completely impenetrable by *R. flavipes*. Below 1.4 mm the rate of tunnelling increases continuously with decrease in particle size. For example, in one experiment over ten days groups of 25 workers tunnelled 0.5 cm through particles 1.4 to 1.18 mm, 4.5 cm through particles 1.18 to 1.0 mm, 7.0 cm through particles 1.0 to 0.85 mm, and 8.5 cm through particles of 0.85 to 0.71 mm. With finer particle sizes the termites penetrated 10 cm in 6 days with particle sizes from 0.71 to 0.5, in 4 days with particle sizes 0.5 to 0.25, and in less than one day with particle sizes below 0.25 mm. Termites were unable to use particles from 1.4 to 1.0 mm for shelter tube construction. Shelter tubes were built slowly and were poorly made when termites were provided with particles ranging in size from 1.0 to 0.71 mm. Shelter tubes were constructed most rapidly (1 cm/day/500 termites) with particles from 0.71 to 0.075 mm. Shelter tubes were most uniform in shape when constructed of particles from 0.25 to 0.075 mm. These experiments suggest that termites probably prefer fine sandy soil (.35 to .075 mm). However, the important effects of soil structure on oxygen availability and soil microorganisms are not known.

Soil particle size also has important effects on water retention and free water availability. Imbibition of hydrostatic water from the soil and water transport have been studied. Termites are able to regulate the microhabitat by transporting water in the crop and regurgitating it for moistening soil and wood. Substantial volumes of water may be involved. Volumes of approximately 10 nanolitres per worker have been measured. Ten water transport trips per worker per day would result in the movement of 100 ml per million workers. Water transport may account for much of the traffic in termite colonies and possibly is critically important in

mixing the population. Population mixing has important implications on the use of transmissible toxicants or pathogens for termite control.

Tree foraging by northern populations of *R. flavipes* was first noted by Hagen (1885) in the Boston, Massachusetts area. The importance of this behaviour in Ontario was re-emphasized by Grace and Cooper (1987). In a study of 17,800 trees in Toronto, Cooper (1981) reported the following rates of termite attack: horse chestnut (19%), silver maple (18%), sugar maple (14%), red oak, Poplar spp., and red maple (5%), Manitoba maple, Norway maple (4%), and locust, white ash, elm, tree of heaven and basswood (2% or less). Over the last year we have noted that tree inspection for the presence of external mud shelter tubes is a rapid and effective method of determining the presence of termites on a property. This method was also used to rapidly identify the location of termites on one infested block in Winnipeg in August 1991. Recently numerous observations of shelter tubes on red pine trees have also been made. Shelter tubes are more likely to occur on older trees and trees with rough bark texture. Studies of orientation responses of the termites to wood extractives of various species also indicate the presence of chemical attractants in horse chestnut and repellents in tree of heaven (Grace, 1991).

Aggregation and Mass Trapping of Termites

As individuals, termites are small delicate insects that are easily killed. As a superorganism of millions of sterile workers, thousands of soldiers, and dozens of reproductives occupying a diffuse network of subterranean galleries over a few thousand square meters they are most difficult to kill. The genus in question, *Reticulitermes*, is particularly difficult because of its ecological adaptation as a *xylophagous forager*. Many other kinds of subterranean termites that harvest renewable cellulose resources (such as grass, humus, dung or fungus combs) from a fixed territory are able to establish a permanent nest or mound. It is relatively easy to control termites that build mounds and conspicuous nests. Xylophagous foragers, however, rely on non-renewable, isolated pieces of surface dead wood. The size and dispersion of this food resource, especially in temperate northern parts of the world, is such that their best strategy is never to invest effort in nest-building, but instead, to always be foraging and searching for new resources so that they can move as resource items are consumed. It is also to their advantage to have numerous, scattered, interconnected feeding sites so that they move from place to place adjusting to the ever changing conditions of temperature, soil moisture, and predation from ants. These ecological adaptations have made the control of subterranean termite colonies a challenge that has defied the creative efforts of researchers for decades. It now seems that the key to controlling such colonies is to develop a *delivery system*. With an effective delivery system a variety of toxic or physiologically active materials might be used to kill the colony.

Mass-trapping and direct treatment of the trapped termites appears to be an effective means of delivering toxicant. A relatively recent innovation in applied termite studies has been the development of various traps which employ corrugated cardboard as a food source and matrix for aggregating and harvesting large numbers of termites (La Fage et al, 1983; French and Robinson, 1985; Grace, 1989; Myles and Smith, unpublished). Using traps containing two small cardboard rolls (15 X 4 cm) Grace (1989) reported average collections of 2,612 termites per 3-10 day interval, with maximum catch of 7,622 and total catch of over 200,000 within one 15 day period. Using larger cardboard rolls (15 X 30 cm) placed in PVC shafts 0.5 to 1.5 m deep, we have been collecting an average of 9,200 termites per trap, frequently with 15,000 to 20,000 per trap and with a maximum of 42,542 from one trap! Through the use of cardboard roll traps we were able to trap 526,000 termites in a three week period from our field site in Kincardine, Ontario. In short, it is now possible to trap hundreds of thousands of termites from single colonies within a few weeks.

We have now devised and installed various "trapping system" consisting of rolls of corrugated cardboard of various sizes, in various arrangements, interconnected with buried fibre tubing. The performance of traps is quite variable from site to site, depending on a great number of uncontrollable variables in the urban environment. Refinement of trap system designs will be an ongoing area of research.

Trap-Treat-Release Approach to Colony Annihilation

The centrepiece of our research is to develop a Trap-Treat-Release (TTR) technique for killing whole colonies of subterranean termites. This concept evolved out of previous research on the Bait Block method and mark-release-recapture studies. The technique involves trapping several hundred thousand termites from buried rolls of cardboard. The trapped termites are treated in the laboratory with small doses of chemicals, insect growth regulators, nematodes, or by physical means. A massive number of treated termites are then released back into the colony. A sudden, massive, widespread mortality sets in throughout the extensive soil tunnel network about one to two weeks after release. The growth of saprophytic and pathogenic microorganisms on the dead will disrupt colony homeostasis. Trap-treat-release cycles could be repeated, if necessary, until the whole colony dies.

By the Trap-Treat-Release approach, trapped termites can be treated under controlled conditions. This creates the opportunity for methods of treatment and types of treatment which have never before been contemplated for termites. For example, cuticular dusting or direct topical application of materials is possible. The Trap-Treat-Release technique is a method of quickly treating a large proportion of the colony within a much shorter period of time than is possible by gradual free-choice feeding at baits. Trapping and treating, though somewhat labour intensive, is a far superior *delivery system*. It is necessary that the lethal effect have a delayed onset so that the treated termites disperse back into the colony. With an effective delivery system, a wide array of *delayed-action* lethal treatments might be used, for example: slow-acting toxicants, insect pathogens, parasitic nematodes, insect growth regulators, or physical treatments such as radiation. Various treatments could also be used in combination. In time, the trap-treat-release technique should be perfected as a routine pest control procedure. Alternatively, trapping, on its own, might become sufficiently perfected that it could become possible to exhaustively trap whole termite colonies out of the ground.

Delayed-Mortality Inducing Treatments

Borate compounds are the primary materials that we have investigated so far (Grace, 1990; Grace & Abdallay, 1991, Grace 1991a,b). Several tested compounds applied as cuticular dusts are effective including: disodium octaborate tetrahydrate (Tim-Bor®), barium metaborate (Busan 11-M1), zinc borate, and boric acid. Sodium borate (borax) was not effective. In experiments in which termites were on filter paper in petri dishes, Effective Lethal Ratios (ELRs) of dusted to undusted termites were at least 1:16. Realizing that in the field the ELR will be affected by the loss of dust from the treated individuals as they disperse through the soil tunnels, we decided to evaluate the ELR after various periods of dust loss. To assess the dust loss we have conducted an experiment in which the dusted termites are placed on moist sand for various time periods prior to transferring them to a group of untreated termites. Even after 24 hours of isolation on moist sand the termites carried enough Tim Bor dust to kill untreated termites at a ratio 1:5. This suggests that Tim Bor dust would be an effective toxicant for a Trap-Treat-Release approach if we can trap 20% of the population. As the mark-release-recapture study suggests we can trap around 10% of the colony. Next we studied the serial transmission of Tim Bor. This was done by exposing an initial group of untreated termites to an equal sized group of dusted termites. After one day the exposed termites were transferred to a new group of untreated termites. This was done for a series of 5 days. The experiment showed that after the first transmission by grooming (termites avidly lick each other) there follows one more effective lethal serial transmission, presumably via trophallaxis of gut content. Thereafter no further lethal transmission in the series occurred.

In recent experiments using Tim Bor, we have tried to improve the adhesion of the dust to the cuticle using a nontoxic adhesive marker pen solutions (Sanford, Sharpie® permanent markers). The marker solution has excellent adhesive properties however we were unable to effectively mark and dust large numbers of individuals. We next experimented with spray paints. Depending on rate of application, several spray paints were found that were non-toxic and effectively aided the adhesion of dust. Two or three cycles of spraying and dusting result in maximal loading. We selected a fluorescent orange because this also conveniently marked the treated individuals. Using this method we have achieved effective lethal ratios of 1:20 in petri dishes and 1:10 in soil tunnel arenas which simulate field conditions. Although dusting-spraying is effective and thousands can be treated within

minutes, we are now trying to improve treatment by formulating the toxicant directly in an adhesive aerosol spray.

An alternative to the use of insecticides is the use of microbial agents. Zoberi and Grace (1990a) isolated 40 fungal species from *R. flavipes* field material. These included several facultative pathogens and a virulent strain of *Beauveria bassiana* (Zoberi and Grace, 1990b). Recently, we have also initiated studies with Dr. Martin Hubbes of the Faculty of Forestry at the University of Toronto on various nematodes that are pathogenic to subterranean termites. In collaboration with Dr. David Levin of the Department of Biology, York University we are undertaking preliminary investigations of several entomogenous viruses. Several non-occluded forms of nuclear polyhedrosis viruses and one entomopox virus have been found that infect and kill *R. flavipes*.

Direct exposure of termites to physical treatments is possible in the Trap-Treat-Release approach. Physical treatments are of considerable interest because they do not produce immediate behavioral impairment and do not burden the insect with carrying a chemical load (allowing the termites to disperse widely from the release point). Physical treatments might be used alone or in conjunction with transmissible chemical or microbial agents. We have investigated various physical treatments as methods of inducing delayed mortality. Short wave UV (9 watts) induced 100% mortality in 9 days after 2-3 minute exposure. With gamma radiation from a ^{60}Co source LD₅₀s from 3 to 28 days were obtained with exposures to 3064 to 383 Rads. Portable gamma emitters with ^{60}Co and ^{137}Cs pellets required 1 and 2 hours of exposure time respectively to induce 100% mortality in 12 days. Termites were X-rayed with a portable 200 KV unit which required only 25 minutes exposure to induce 100% mortality in 10 days. UV is least hazardous form of physical treatment and requires the shortest exposure time. However since gamma and X-rays could penetrate cardboard and soil they could be used in an "aggregate and irradiate" strategy. A portable X-ray or gamma emitter could be mounted on a small wagon and driven to any location. It could then be used to irradiate the aggregated termite *in situ* in the aggregation traps without even removing the trap from the ground. Two or three exposure cycles might be enough to kill a colony. This would entail relatively small labour costs, and would leave no toxic chemicals in the soil.

Benefits of Research

Present methods of control are property-specific and heavily dependent on large gallonages of persistent pesticides applied as a chemical barrier in the soil around an infested structure. This method is usually effective for protecting a given structure (although re-treatment is often required). However, the chemical barrier method is almost never effective in killing the termite colony which simply moves from the treated site to an adjacent untreated property. By the Trap-Treat-Release technique it will be possible to eradicate isolated pockets of termite infestation in Ontario municipalities rather than simply protecting properties one at a time. Eventually it may be possible to eradicate termites block by block to re-establish "termite free zones" in Metro Toronto.

With further development, the Trap-Treat-Release technique will become an integral component in urban pest management. Effective development of the Trap-Treat-Release method will substantially reduce or eliminate the amount of persistent pesticide used for subterranean termite control. By reducing the need for termiticides, it will dramatically lower the load of persistent toxicants in the urban environment. The reduction of termiticide treatments will also decrease the long-term health hazard to residents of termiticide-treated homes, arising from lingering vapours in in-door atmospheres and from accidental contact with treated soil. It will also reduce or eliminate the health hazards to applicators of termiticides arising from spills and misapplications.

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The Development of Reliable Treatment Systems for Milkhouse Washwater; M. Anderson and P.H. Groenvelt, University of Guelph, Guelph, Ontario

Introduction
Research conducted by the Upper Thames River Conservation Authority has identified the improper disposal of milkhouse wash water as one of the most serious sources of agricultural pollution in the province, amounting to over 200 tonnes of phosphorus entering rivers annually in Southern Ontario alone. This is approximately 12% of the estimated load to Lake Erie from agricultural land in Ontario, assuming all this phosphorus reaches surface waters. Compounding this is the fact that 63% of this phosphorus is soluble reactive phosphorus, and thus much more available for algal growth than phosphorus pollution from run-off (Miller et al., 1987). In addition to phosphorus, this effluent usually contains large amounts of residual milk which is a strong source of BOD and bacterial pollution.

A large part of the problem is that farmers who do not have liquid storage facilities currently lack a reliable method of disposal. In the past, the Ontario Ministry of Agriculture and Food has recommended the installation of septic tank-treatment trench systems on such farms. However, a large proportion of these systems which were installed have failed, and as a result have acquired a reputation within the farming community as being quite unreliable.

The primary goal of this research project is to develop simple modifications to the milkhouse septic tank system design or on-farm practices which will render these systems reliable for at least 25 years.

In order to achieve this goal, the direction this research project has taken in the last six months is not so much to modify the treatment trench disposal system, as to modify the characteristics of the effluent so it will be less prone to cause failures of these systems.

Farmer survey:

A mail survey was sent out in late November, 1990, to 128 farms which had installed milkhouse treatment trench systems under the OSCEPAP II program. Of the surveys sent out, 89 responses were received. The aim of this survey was to identify factors which were contributing to the failure of the treatment trench disposal systems and to determine their relative importance. Included in the survey were all the conceivable factors which might affect the lifespan of these systems, including: soil type, water use, milk spills, septic system design, length of milk pipeline, etc. An example the survey is found in appendix I.

Survey Results:

Number of surveys sent out: 128
Number of returns: 89
Number of case studies: 104
(many surveys provided information on more than one system)

Number of systems still functioning: 65

-which are diverting rinse water: 28
-which are not diverting rinse water: 29
-which are not using a pipeline: 8

Number of systems which have failed: 39

-which were diverting rinse water: 19
-which were not diverting rinse water: 18
-which were not using a pipeline: 2

Details of disposal system history:

For systems still functioning

Ave. lnth. pipeline 210 feet
Ave. # of cows 36
Ave. est. water use 129 gallons
Ave. drain time 6.8 minutes
Using air injectors 54%
Any milk spills? 26%

For failed systems

181 feet
36
106 gallons
5.6 minutes
54%
33%

Ave. vol. settling tks. 722 gallons
With baffled outlets 78%
Ave. length of trench 264 feet
Ave. yrs. btwn. pumpings 2.6 years
Ave. date of install. 1987
Requested survey results 74%

475 gallons
62%
223 feet
1.7 years
1980
96%

Statistical analysis was carried out on those case studies involving a failed system. A multiple regression was set up involving the following factors:

- water use
- Number of settling tanks
- volume of settling tanks
- length of weeping bed
- clay content of soil
- milk spills
- diversion of rinse water
- hydraulic loading rate (water use/length of weeping bed)

The response used in this analysis was the lifetime of the system in years. The only factors which appear to significantly affect the lifespan of these systems is the diversion of rinse water and hydraulic loading rate. Essentially this means that if farmers use less water and exclude the rinse water from the septic systems, these systems will work significantly better.

The Effect of Diverting Rinse Water on Effluent quality:

A central hypothesis of this research is that the diversion of pipeline rinse water will reduce the formation of a thick soil clogging mat in the weeping bed. Research regarding household weeping bed systems has shown that the extent to which a clogging mat develops is accelerated by effluents of higher organic strengths and by higher hydraulic loading rates (Jones and Taylor, 1965; Laak, 1970; Siegrist, 1987). More specifically, the development of this mat seems to be directly related to the cumulative loading of BOD and total suspended solids (Laak, 1970; Otis, 1985; Siegrist, 1987).

In January, 1991, an experiment was conducted on a farm east of Peterborough, Ont. in order to determine what effect the diversion of rinse water would have on effluent quality and therefore on the organic loading rate to the weeping bed.

Since 1978 this farmer had installed two weeping bed systems and two dry wells in order to treat his wash water, none of which lasted more than one year. He was currently allowing the settling tank to overflow and run down into a gully. He had also never diverted his rinse water.

We determined that since he had recently installed a semi-solid manure system, a solution in his case involved only the re-routing of the drain in his milkhouse sink to drain into his manure gutter. However prior to this, he consented to begin diverting the pipeline rinse water in order to investigate the effect this would have on the quality of the effluent in the settling tank. Weekly samples were taken from the tank for a period two weeks before to two weeks after he began diverting the pipeline rinse water. Samples were analyzed for BOD and TSS with results shown in fig 1 and 2 in the appendix. TSS showed a 85% reduction within one week of diversion and an 89% reduction over two weeks. Similarly, BOD values dropped by 42% within one week and 74% within two weeks.

While this farmer is now putting his milkhouse wash water into liquid manure storage, he plans to continue to divert the pipeline rinse water as he feels it is good feed for his calves.

Water use Studies:

It is well documented in scientific literature relating household weeping bed systems that a reduction in the hydraulic loading rate will reduce the likelihood of system failure, independent of the organic loading rate (Seigrist, 1987; Sharpe et al., 1984). This is presumably due to more efficient biodegradation of organic matter in the weeping bed when water is not continuously ponded in the trenches.

Unfortunately, there is very little literature relating to water use by modern dairy operations. In addition, from the survey results, there is no correlation between estimated water use and pipeline length, pipeline diameter, number of cows or even the number of wash cycles per day.

There are two possible explanations for this lack of correlation: either farmers are very bad at estimating their water use or there is another factor which dominates water use which was not included in the survey.

In addition to improving the function of milkhous weeping bed systems, reducing water use would have several other benefits which would be attractive to farmers. Approximately 2/3 of the water farmers use for pipeline washing is hot water. A reduction in water use would bring a considerable economic savings in terms of hydro use and also in terms of cleaning chemical use. Hydro and chemical costs typically range into the thousands of dollars per year per farm.

In order to investigate the factors which affect water use, several milkhouses have been instrumented with meters to measure hot water use, total water use and hydro use by the milkhous hot water heater. Farmers involved in these studies have agreed to read these meters every day and have been encouraged to experiment on their own to develop simple ways to reduce water use.

The water meters used were Neptune Bronze 1/2" water meters obtained from Canada Valve in London, Ont. and rated accurate within 1%. The hydro meters were obtained from Waterloo North Hydro for a nominal fee and are also rated accurate within 1%.

Several mechanical modifications will be investigated for their effect on water use. Specifically, "air injectors" for pipeline circulation, and "wash manifolds" to more efficiently use the water in the milkhous sink, are both commercially available and are claimed to reduce the amount of water used for pipeline cleaning.

Water Use Study Results:

At the time this report was written, three farms were participating in this study with two others as yet to be instrumented. Results for two of the participating farms are seen in figs. 3 and 4 in the appendix.

Substantial water savings have already been achieved on two of the participating farms. Bill Irwin had for many years been using approximately 150 l/cycle which would amount to approximately 1300 litres of water per day. During a farm visit in Nov. 1990, his excessive water use was discussed and he adjusted the level to which the water in his sink filled. This simple adjustment, involving only a length of hose in his milkhous sink, reduced his water use by over 34%. In the spring of 1991, a similar adjustment was made resulting in a further 47% reduction, for a total water use reduction of 65% or 850 litres of water/day.

From measurements of hydro consumption/l of hot water, this will result in an energy savings of 11,500 KWH/yr. amounting to approximately \$800/yr.

Ron Forbes, another farmer who is participating in this study, has cooperated in redesigning a milkhous sink which has reduced his water use by approximately 40%. This will result in total water savings of 275 litres of water/day and hot water savings of 62,500 litres/ yr. Energy savings will amount to approximately 3,050 KWH/yr. or \$210/yr.

In addition to looking at general water use and investigating methods to achieve reduction, we will also be comparing farms for energy efficiency as some farms are using heat exchangers to recover heat from the bulk tank. Results for energy consumption in relation to hot water use are given for two of the participating farms in fig 5 and 6. It is apparent from these figures that heat exchangers offer substantial energy savings. Ron Forbes, who has a heat exchanger is using 47.7 KWH per cubic metre of hot water. Bill Weiker, who is not using a heat exchanger, is consuming 75.0 KWH per cubic metre which is 36% less efficient.

Research in this area will continue in the following year.

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POTENTIAL METHODS OF REVEGETATING THE KAM-KOTIA TAILINGS SITE, TIMMINS. Keith Winterhalder, Biology Department, Laurentian University, Sudbury, Ontario P3E 2C6.

The Kam-Kotia mine/mill complex is located 15 km northwest of Timmins. Following closure in 1972, the property reverted to the Crown without rehabilitation. The area requiring revegetation is a sulphide-rich, acid-generating tailings deposit covering 275 hectares, including impounded areas where the tailings form a consistently deep layer and non-impounded areas where the thickness of tailings is highly variable. Acid mine drainage from the tailings has a pH of around 2.5, and is high in arsenic, zinc and copper.

Prior studies (e.g. that by the D. Comrie Consulting Ltd (1987)) recommended sophisticated and costly solutions. In 1990, the author was funded by the Kam-Kotia Steering Committee to carry out a number of small-scale trials employing varied "low-tech", low-cost revegetation techniques.

MATERIALS

a. Growth media and base materials

Pit-run gravel was used both as a growth medium and as a base material. "**Loam**" - an approximately 50:50 screened mixture of "red loam" and "black loam" - was used as a growth medium (although **red loam** was also used in some treatments).

Peat moss was either used on its own as a growth medium, or else was mixed into the upper few centimetres of the gravel, where its function was to improve moisture-retaining capacity and to provide both a nutrient source and a nutrient-adsorbing material. **Bog peat** served both as an oxygen-barrier and as a base for living bog-sods collected at the same bog.

b. Neutralizing agents

Ground Dolomitic Limestone contained 2% calcium and 12% magnesium with a CaCO_3 -equivalent of 101%. It was ground such that 5.4% passed a 100 mesh screen and 100% passed a 10 mesh screen. Unless stated otherwise, the application rate was 4 t/ha (1.6 kg per 4 m² plot). **Sewage Incinerator Ash** is a by-product of a Toronto sewage treatment plant, in which the sludge removed during the treatment process is burned. The major constituent of the ash (38%) is a calcium magnesium phosphate ($\text{Ca}_7\text{Mg}_2\text{P}_6\text{O}_{24}$). Unless stated otherwise, the application rate was 2 t/ha (0.8 kg per 4 m² plot). **Marl** was obtained from the Pup lakes (formerly Twin lakes) area in west-central Thorneley township, District of Temiskaming. It contained approximately 87% CaCO_3 and 2.2% MgCO_3 . Unless stated otherwise, the application rate was 4 t/ha (1.6 kg per 4 m² plot).

c. Fertilizers

A 6-24-24 agricultural-grade fertilizer was used at a rate of 400 kg/ha (160 g/4 m² plot)

d. Plant material

i. **Seeds** The grass-legume seed mix had the following constitution*:

<i>Agrostis gigantea</i>	Redtop	10%
<i>Festuca arundinacea</i>	Tall Fescue	30%
<i>Phleum pratense</i>	Timothy	20%
<i>Poa compressa</i>	Canada Bluegrass	10%
<i>Poa pratense</i>	Kentucky Bluegrass	20%
<i>Tritolium hybridum</i>	Alsike Clover	10%

* Seed of Birdsfoot Trefoil (*Lotus corniculatus*) was not available at the time, but will be dormant-seeded onto the plots in the late fall of 1991.

The seed mixture was sown at a rate of 30 kg/ha (12 g/ 4 m² plot), and lightly raked into the plot surface.

ii. **Transplanting stock**

The following plant material was collected from acid, metal-contaminated soils in the Sudbury area, where there was some evidence of their acid- and metal-tolerance:

Tufted Hairgrass (*Deschampsia caespitosa*)

Redtop (*Agrostis gigantea*)

Wool Sedge (*Scirpus cyperinus*)

Dwarf Birch (*Betula pumila*)

All samples were collected with a large portion of native soil attached to the roots.

METHODS

a. Seeded Plots were set up on the well-drained North Impounded Tailings. Their dimensions were 2 m x 2 m, with a 0.5 m sloped buffer zone. They were set up in triplicate, using a randomized block design. Plots fell into three categories:

i. **Direct amelioration** of tailings. Current knowledge of the chemistry of the tailings strongly suggests that direct revegetation, however effective the neutralizer, will not succeed in the long run. It was felt, however, that this hypothesis should be checked experimentally. Dolomitic limestone and marl were applied at 20 t/ha and ash at 10 t/ha, respectively, and raked into the surface of the tailings.

ii. Use of a **gravel covering or overburden**. The rationale for the use of coarse gravel as a covering for the tailings is based on its porosity. Upward movement of acid ground-water by capillary action is low, and acid ground-waters are easily washed out by rain. Disadvantages of this growth medium are the low nutrient content and nutrient-retaining capacity, and the low moisture-retaining capacity. However, past experience has shown it to be an effective covering, both with (Spires, 1975) and without (Michelutti, 1974) a loam covering. The plots were constructed with gravel alone, gravel topped with commercially available peat moss which was worked into the top 5 cm of the gravel, gravel topped with locally-obtained red loam and gravel topped with the "loam" described above. Depth of both gravel and covering (where employed) were varied. In each case, one of the three neutralizers was raked into the surface, along with the standard fertilizer.

iii. Plots based on a **loam covering**. In each case, one of the three neutralizers was raked into the surface, along with the standard fertilizer.

b. Transplant plots fell into the following categories:

i. **Direct transplants**. Potentially tolerant Sudbury plants were transplanted, in a block of their own soil, into transects having three treatments - untreated tailings, limed tailings and gravel over tailings.

ii. Plots involving the use of a living **bog-sod** cover. In these plots, blocks of bog peat, complete with living vegetation, have been placed on top of the tailings. In this case, no neutralization, fertilization or seeding was carried out since the intention was to have the bog plants survive in the peat block, which will itself impede oxygen penetration and tailings oxidation. Native peat from the bog was also used to form the 0.5 m shoulder.

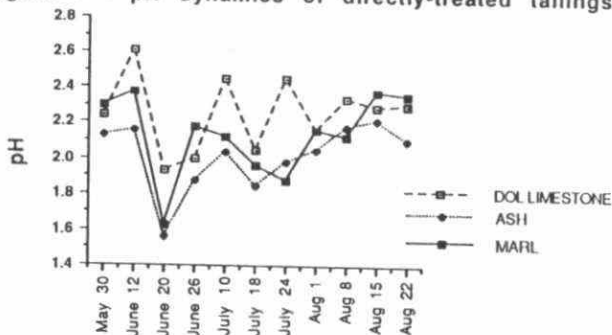
c. Monitoring. A photographic record of the plots was obtained several times during the summer of 1991. Soil samples were obtained from the surface 10 cm of each plot on a weekly basis, and analysed for pH and moisture content. Biomass was determined in August by harvesting the vegetation on 50% of each plot, drying it and weighing it. Cover material samples were retained for possible future metal analysis.

RESULTS

a. Seeded Plots

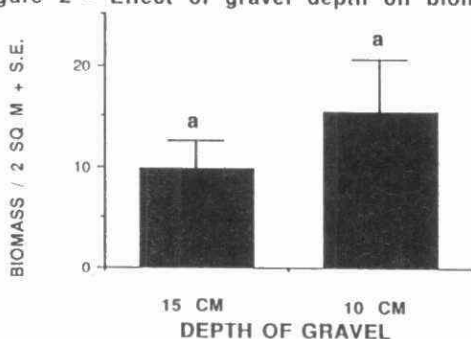
i. **Bare tailings**. As predicted, there was no plant survival on directly-treated plots, although there was some germination and short-term growth. By the time monitoring began in May following treatment the previous fall, the pH was already very low, and, despite fluctuations, it remained so (Figure 1).

Figure 1 - pH dynamics of directly-treated tailings



ii. **Gravel-covered tailings.** On gravel-covered tailings, biomass production was low, but survival was, in most cases, reasonable. As shown in Figure 2, biomass production was somewhat less on 15 cm of gravel than on 10 cm, but not significantly so.

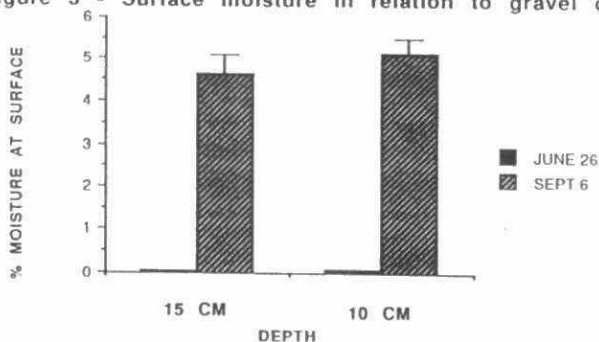
Figure 2 - Effect of gravel depth on biomass production



Bars bearing the same superscript are not significantly different at the 5% probability level.

The reduced growth on the deeper gravel was presumably due to greater exposure, both in terms of drying and of wind-borne tailings abrasion. Figure 3 shows comparative moisture levels at a dry time and a moist time, respectively. Moisture content is consistently higher in the 10 cm gravel plots, but the difference is not significant statistically.

Figure 3 - Surface moisture in relation to gravel depth



Figures 4 & 5 show the changes in pH that occurred in the top 5 cm of the gravel plots. pH dropped sharply at first, but levelled off between pH5 and pH6. There seemed to be very little difference in pH dynamics between the 15 cm and 10 cm gravel plots.

Figure 4 - Surface pH dynamics in 15 cm gravel plots

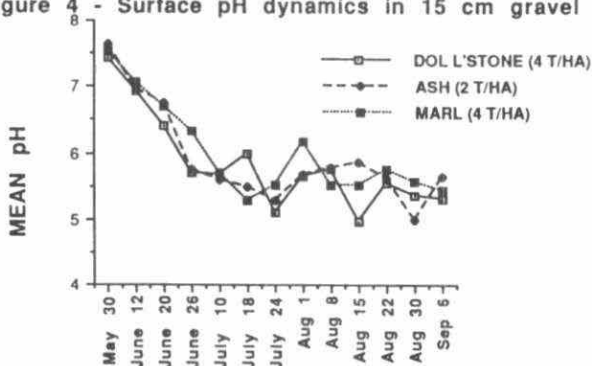
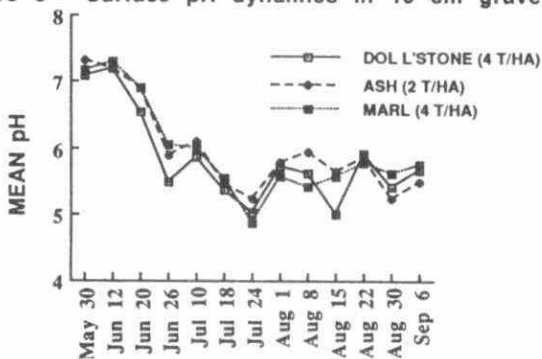
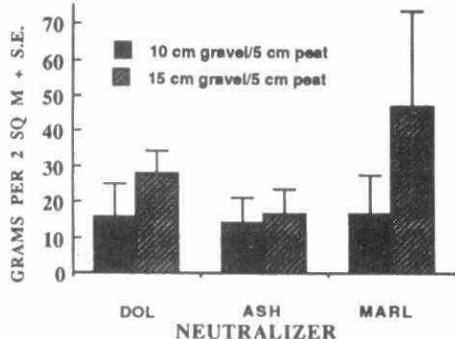


Figure 5 - Surface pH dynamics in 10 cm gravel plots



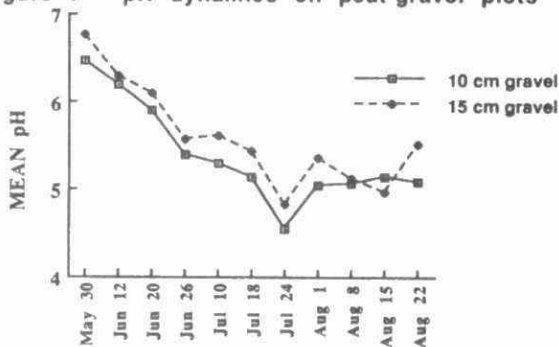
iii. **Gravel-covered tailings with peat worked into surface.** Contrary to the situation described for pure gravel, growth was better on 15 cm of gravel with 5 cm peat worked into the surface than on 10 cm with the same amount of peat (Figure 6).

Figure 6 - Biomass on peat mixed into gravel



It is possible that the greater proximity of the peat's capillary-enhancing properties to the tailings surface led to great upward movement of acid in the shallower gravel, a hypothesis that is supported by the pH trend shown in Figure 7.

Figure 7 - pH dynamics on peat-gravel plots



iv. **Loam.** On loam-covered tailings, a gradual drop in pH occurred, but only in the plot with a 5 cm cover did the pH drop below 6.0. This is illustrated for the marl treatments in Figure 8.

Figure 8 - pH dynamics on marl-treated loam

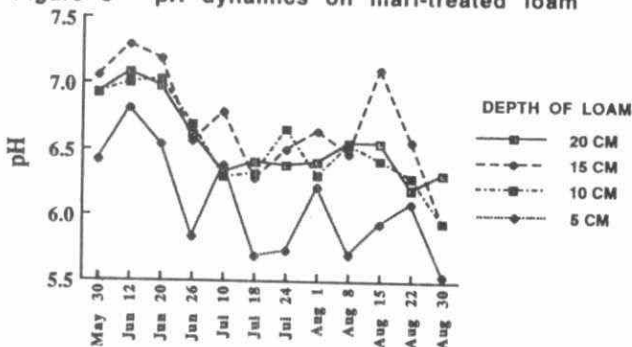
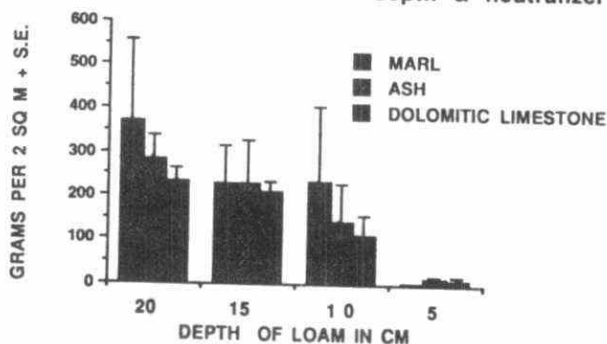


Figure 9 summarizes biomass production on loam.

Figure 9 - Biomass v loam depth & neutralizer

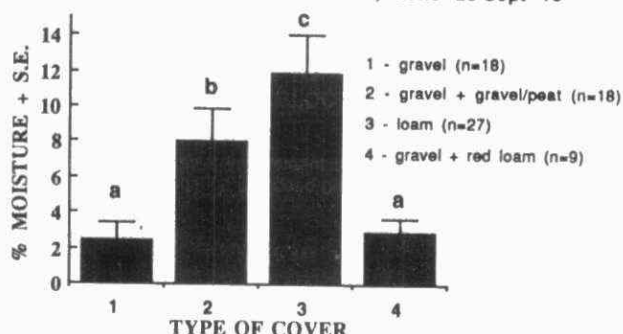


It is clear that the thickness of a loam cover should not be reduced below 10 cm. Effectiveness of neutralizers appears to follow the sequence of marl>ash>dolomitic limestone, but it must be remembered that the marl contains phosphorus, and could be playing a fertilizer role.

v. General

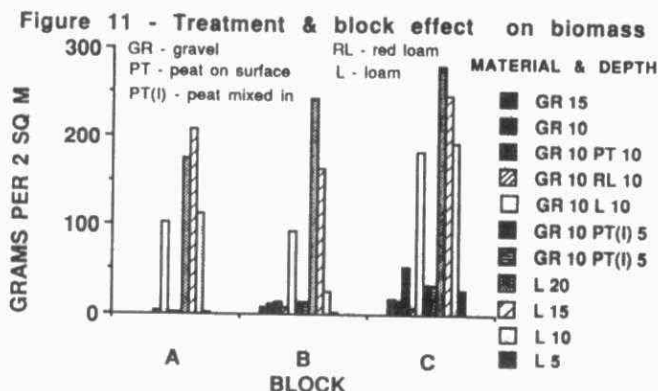
Figure 10 shows mean moisture levels of the different covers for most of the summer period. Not surprisingly, loam has the best moisture-retaining properties.

Figure 10 - mean surface moisture, June 26-Sept 13



Bars with the same superscript do not differ significantly at the 5% probability level.

From Figure 11, it is clear that Block C, which is located within the shelter of dead treetops, has a more favourable environment than the other blocks. It is also clear that a 15-20 cm layer of loam is likely to give the best biomass, with 10 cm of gravel covered by 10 cm of loam the second-best.



Future observations on the upward-movement of acidified tailings water will probably determine whether the gravel-loam combination is indeed preferable to the loam alone.

b. Transplant Plots

i. Direct transplant plots. No quantitative data are available at this time, but it can be stated that there was a good survival rate overall. Dwarf Birch was most successful on the North Unimpounded site, and Wool Sedge on the moist South Unimpounded site. Tufted Hairgrass and Redtop did well in all sites. A quantitative assessment will take place in 1992.

ii. Bog Sod Plots. All bog species remained vigorous.

CONCLUSIONS

While one year is too short an experimental period to be able to draw firm conclusions, it appears that it may be feasible to use of a cover material to revegetate the well-drained portion of the Kam-Kotia tailings. Preliminary results suggest that a loam covering or a gravel covering topped with loam would be most effective, but at least one more season of observation will be required to determine the relative vulnerability of different cover combinations to upward movement of acids.

The use of metal-tolerant transplants from the Sudbury area also shows some promise. The bog-sod transplant approach also shows promise, but it is probably neither economically feasible nor conservationally desirable to use this technique on a large scale, unless plans to develop the horticultural peat industry in Northern Ontario come to fruition.

ACKNOWLEDGEMENTS

A number of individuals have assisted in this project, and the author wishes to acknowledge the considerable contribution of those most recently involved, viz. Daniel Archambault, Derek McHale, Allan Petryna and Bryan Tisch.

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**SOLID WASTE PRODUCTION AND MEASUREMENT IN
LAND BASED CULTURE OF RAINBOW TROUT (*Oncorhynchus mykiss*)**

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ABSTRACT

Solid waste is produced in land based trout culture in the form of uneaten and undigested feed. Undigested feed is equivalent to approximately 0.27 grams dry matter faecal waste per gram of dry pelleted fish feed. At this rate of production, distribution between suspended and settled solid wastes is variable with total solids equivalent to concentrations of 100 ppm or less in the flow through fish rearing tanks. Higher concentrations occur when tanks are cleaned, and generalized estimate of solid waste quantiles due to uneaten food are not known. At concentrations of 100 ppm and less, individual solid particles settle initially as discrete particles without interference between particles in Type I settling. Subsequent Type II settling occurs as concentrations increase with depth, followed by Type III and Type IV settling when compression of settled solids produces concentrations of 50,000 ppm. Four types of settling were investigated using fresh faeces from 300 gram (0.66 pounds) rainbow trout held at a stocking density of 35 kilograms per cubic meter (2.2 pounds per cubic foot). Type I and Type II settling were observed to determine that 90% of initial concentrations, at 30 ppm and less, settled from suspension in ten minutes or less. For similar initial concentrations Type II settling was observed in Imhoff cones and resulted in flocculated concentrations in excess of 20,000 ppm within 20 minutes. Subsequent Type III and IV settling produced concentrations in excess of 50,000 ppm within one hour. The preceding experiments provide the basis for current investigation of the settling characteristics of solid faecal waste produced by rainbow trout as they grow from an initial size of 25 grams (.9 ounces) to 250 grams (0.6 pounds). Results will be applied to the development of design guidelines for sedimentation tanks required at different stages of fish growth. Both ongoing studies and prerequisite investigations are funded by The Ontario Ministry of Agriculture and Food and The Ontario Ministry of The Environment.

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BACKGROUND AND OBJECTIVE

Undigested feed and uneaten feed appear as solid waste in land based trout culture. The quantity of uneaten feed differs with feeding method but generalized estimates of solid waste due to uneaten feed are not available. Undigested feed is equivalent to 0.26 to 0.28 grams dry matter faecal waste per gram of dry pelleted fish feed (Willoughby et al., 1972; Windel et al., 1978; Butz and Vens - Cappell, 1982; Clark et al., 1985; Farrell, 1990).

Waste solid particles that are heavier than water can be removed by gravity settling in controlled settling zones (Wheaton, 1977; Metcalf and Eddy, 1979; Boersen and Westers, 1986). The primary purpose of controlled settling is to produce a clarified effluent from the controlled settling zone, but the production of a sludge that can be easily removed and disposed of is also necessary.

In the case of municipal sewage, design of settling works is based upon theory about four distinctly different settling processes that occur in the treatment of municipal sewage. Individual particles in dilute suspensions settle as discrete particles, in Type I settling, without joining of individual particles to form coalesced flocs. Flocs have greater mass and higher settling velocities than individual particles and settle more quickly as attributed to Type II settling.

As coalesced flocs settle downwards, concentrations increase and interference between flocs occurs at concentrations between 2600 and 6600 parts per million (Fair et al., 1968). At such concentrations settling velocities decrease and interference between flocs results in mass settlement distinctive of Type III settling. In Type IV settling the concentration of interfering flocs increases, structure is formed and compression of settled solids follows as solids are continuously added to form concentrations in excess of 50,000 parts per

million.

Concentration of solid waste in the flow through trout rearing operations is variable over a representative range of 0 to 104 parts per million having an average concentration of 7 parts per million (Liao, 1970). At such low concentrations Type I settling would occur during initial stages of the settling process.

Thomson (1986) and Stechey and Trudell (1990) investigated the settling characteristics of rainbow trout solid waste mixed in suspensions at initial concentrations an order of magnitude greater than the initial concentrations observed by Liao in 1970. The studies by Thomson and Stechey demonstrate the occurrence of Type II and Type III settling of solid waste from rainbow trout. Evidence of Type IV settling is available from studies by Boersen and Westers (1986) who report settled solid waste concentrations in excess of 50,000 parts per million typical of Type IV settling.

The preceding independent studies show that the four settling processes in municipal sewage occur in settling of solid waste from rainbow trout. However, the characteristics of process in each of the four types of settling of rainbow trout solid waste are imperfectly understood at this time. Also current studies do not include application of proven experimental methods to the analysis of overall process in settling of solid waste from rainbow trout.

It is not evident that known experimental methods can be successfully applied to the analysis of process in settling of solid waste from rainbow trout. Nor is it clear that existing settling theory can be applied to the design of settling works in land based trout culture.

The objective of this study is to describe a preliminary investigation of Type I, II, III and IV settling of solid waste from rainbow trout using current experimental methods. Experimental results will be interpreted for application of existing settling theory to design of settling works in land based trout culture.

METHOD

Fresh settled faecal solids were collected from rainbow trout (*Oncorhynchus mykiss*) of average size 300 grams (0.66 pounds) held at a stocking density of 35 kilograms per cubic metre (2.2 pounds per cubic

foot). At this density break-down of faecal pellets due to fish movement was not evident. Solids settled to the bottom of the holding tanks and were captured in sampling hoppers at the bottom of the tanks.

Tests for Type I and Type II settling were completed in a settling column 1000 mm (39.3 inches) deep and 150 mm (6 inches) inside diameter. Dilute suspensions at uniform initial concentrations of 15 to 30 parts per million of fresh faeces were sampled at one minute intervals from a sampling port at a depth of 750 mm (30 inches). Conventional methods (Camp, 1946) for the analysis of Type I and Type II settling in municipal sewage were applied to calculate settling velocity of solid particles, at a depth of 750 mm, in relation to the concentration of particles remaining in suspension at a depth of 750 mm (30 inches).

As background to analysis of Type III and Type IV settling, dilute suspensions at uniform initial concentrations of 15, 20, 25, 30 parts per million were settled in one litre Imhoff cones. These tests provided estimates of the volume occupied by the uncompressed settled solids, and their concentration at the transition between Type II and Type III settling.

Guided by results from the Imhoff cone tests, concentrated suspensions at uniform concentrations of 19,600, 20,200, 21,800 and 24,100 parts per million were observed to settle in a 300 mm (12 inch) graduated cylinder. Rate of settling was determined according to observed downwards movement of the interface between "clear" liquid and concentrated solids below the interface. Observations were analyzed according to the method developed by Talmadge and Fitch (1955) for differentiating Type III and Type IV settling of solids in municipal sewage.

RESULTS

In the tests for Type I and Type II settling, standard settling column analysis of dilute suspensions was limited to observations at a single depth of 750 mm. (30 inches). Results were analyzed as proposed by Camp (1946) for municipal sewage, and showed that the concentration of suspended solids decreased systematically with time. Settling was rapid with removal of over ninety percent of suspended particles within ten minutes. The corresponding average particle settling velocity was 0.1 metres per minute.

Consistent with results from the Imhoff cone samples, tests for Type III and Type IV settling were

based upon suspended solids concentrations in the range 19,600 to 24,100 parts per million initial concentration. Results were analyzed as proposed by Talmadge and Fitch (1955) for municipal sewage. For the test range in initial concentration, the initial settling velocity remained constant for approximately three minutes. The observed range in initial settling velocity was 4.3 to 2.1 metres per hour and initial settling velocity decreased with increasing initial concentrations.

After approximately three minutes the settling velocity decreased systematically in all cases. By fifteen minutes the transient settling velocity was reduced to near-zero constant rates that occur during compression of settled flocs.

DISCUSSION AND CONCLUSIONS

In this study, current methods for the analysis of settling in municipal sewage were used to investigate settling in dilute and concentrated suspensions of solid waste from rainbow trout. Settling of dilute suspensions was examined by conventional settling column analysis which is based upon theory about Type I and Type II settling (Camp, 1946). Analysis produced estimates of percentage removal of settled solids in relation to depth and time, and estimates of particle settling velocity. These settling characteristics determine the design of effective settling tanks for removal of solids from effluent of land based trout culture operations.

Settling of concentrated suspensions was analysed according to methods proposed by Talmadge and Fitch (1955) for municipal sewage. This analysis is based upon theory about Type III and Type IV settling and establishes relationships between settling time and resulting concentration of thickened settled solids deposits. Thickening characteristics of settled solids deposits determine the size of settling tank required to produce optimum concentrations in compressed solids for effective removal.

Results from the study are preliminary as they refer to a specific fish size, stocking density and diet. Within these constraints, current theory about settling in municipal sewage can be applied to determine the settling characteristics of dilute and concentrated suspensions of solid waste from rainbow trout. These settling characteristics determine the basis for design of effective settling works.

According to the results of the preliminary study current experimental methods will be applied to determine the settling characteristics of solid faecal waste produced by rainbow trout as they grow from an initial size of 25 grams (0.9 ounces) to 250 grams (0.6 pounds). Results will be applied to development of design guidelines for solids settling and solids thickening works required at different fish size in land based cultures of rainbow trout.

ACKNOWLEDGEMENTS

The preliminary experimental work reported in this study was completed by fourth year undergraduate engineering students Michelle Lue and Steve Brisson. Both prerequisite studies and ongoing investigations are funded by The Ontario Ministry of The Environment and The Ontario Ministry of Agriculture and Food.

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Field and Laboratory Evidence of *In Situ* Biotransformation of Tetrachloroethene to Ethene and Ethane at a Chemical Transfer Facility in North Toronto¹. David W. Major and Eric W. Hodgins, Beak Consultants Limited, 595 Woolwich Street, Guelph, Ontario N1H 3Y5, and Barbara J. Butler, University of Waterloo, Department of Biology, Waterloo, Ontario N2L 3G1.

INTRODUCTION

A potential real estate transaction initiated a groundwater investigation at a 4.5-acre chemical transfer facility located in North Toronto. The fenced facility is comprised of small office, boiler and weigh scale building, road with turnabout, railway spur line, and above- and below-ground transfer lines (Figure 1). Organic solvents are delivered to the site by rail and transferred by below- and above-ground pipes to the above-ground tank farm and eventually to tanker trucks. Both the previous and current owners of the facility stored and transferred similar organic solvents (methanol, methyl ethyl ketone, vinyl ethyl acetate, butyl acrylate) with one notable exception. The previous owner had stored tetrachloroethene (PCE) at the site approximately 10 years ago. Preliminary groundwater investigations revealed both free and dissolved PCE, and the dechlorination intermediates of PCE had contaminated the groundwater below the facility. The absence of PCE, or its intermediates, between the former PCE storage tank and the spur line suggests that small amounts of free-phase PCE were released during the coupling and uncoupling of railway tanker cars, and not from the above-ground storage tank.

Pumping test data indicated that pumping could effectively contain the dissolved PCE on site, but the hydraulic conductivities of the aquifer were too low for the feasible application of pump and treat strategies to reduce source area PCE concentrations. However, the presence of dechlorination products suggested that anaerobic biotransformation of PCE might be an attenuating mechanism, one that could be exploited in remediating the site if vinyl chloride (VC) was not accumulating. As a result we undertook a field and laboratory investigation to determine if VC was being mineralized or biotransformed to innocuous end products. If VC was being biotransformed, we wished to understand the physical, geochemical, and microbial processes that promote the conversion of PCE past VC. This paper presents our initial field and laboratory data that provide evidence of *in situ* biotransformation of PCE to ethene.

MATERIALS AND METHODS

Details of groundwater and sediment collection, geochemical and geological analysis, microbial analysis and microcosm studies are presented elsewhere (Major *et al.*, 1991²).

Geochemistry Parameters included in the analysis included cation/anion scans, dissolved organic carbon (DOC), metals, alkalinity, pH, Eh, dissolved oxygen (DO), temperature, hydrogen gas, hydrogen sulfide, C1-C3 gases (methane, ethane, propane), volatile fatty acids (notably acetate), chlorinated alkanes and alkenes, and water soluble constituents such as methanol, butyl acrylate and other solvents stored at the site. Groundwater from selected monitoring wells were exhaustively extracted (base/neutral/ and acid) and analyzed by GC/MS. Analysis for cations and anions, alkalinity, hydrogen sulfide, metals, and DOC were conducted by Beak Consultants Analytical Services in Brampton, Ontario. Chlorinated alkanes, and alkenes, ethene, ethane, volatile fatty acids, water soluble constituents and GC/MS analysis were conducted by Ralph Dickhout at University of Waterloo, Department of Earth Sciences, Waterloo, Ontario. Jim Gossett of Cornell University, NY, provided confirmatory chlorinated and ethene and ethane analysis as well as hydrogen gas analysis.

Geology The site geology was assessed using continuous coring techniques in selected locations, drillers logs, and downhole geophysical methods. Core material collected during continuous coring operations was subsampled for grain size analysis and P_{ore} determinations (analysis conducted by the University of Waterloo). Hydrogeological data were obtained from the site by monthly measurements of the piezometric level in monitoring wells, well response tests and drawdown responses during pumping.

Aquifer Sediments. The Waterloo Saturated Sand Sampler (WSSS) was used to secure and retrieve aquifer sediments from an area known to have concentrations of PCE (ISRP 5-1). The sediments were for use in microcosms and microbial assessment. Once the WSSS was brought to the surface, the core liner was removed and quickly cut into 60 to 70 cm lengths, capped, and the liner cut lengthwise with a circular saw. One core was not split but was capped, wrapped, placed on dry ice and express-shipped to the University of Tennessee for phospholipid fatty acid analysis. Split cores were immediately placed into an anaerobic glove box and sterile tools were used to pare off the outermost portions of the core. The inner, aseptic core material was collected and distributed into sterile Whirl-Pak bags which were placed in to anaerobic jars. The jars were sealed, then removed and stored on ice for transport from the site to the University of Waterloo.

Enumeration of Microbial Populations. Sulphate-reducing and methanogenic bacteria were enumerated using a three-tube, most probable number (MPN) procedure using a modified (McInerney *et al.*, 1979) Postgate medium (1984). All culture

¹This is abridged version of a same titled paper to be published in *In Situ Bioreclamation*. Ed. R. Hinchey, Butterworth Publishers, 1991.

²Ibid

tubes were incubated at room temperature for up to 3 months. Methanogens were enumerated in a Zehnder and Wuhmann (1979) medium modified by Zeikus *et al.* (1979). Total phospholipids were extracted from frozen subsurface sediments by a modified single phase chloroform-methanol method as previously described (Robbie & White 1980). Methyl esters of the phospholipids (phospholipid fatty acids, PLFA) were analyzed by capillary GC as described by Robbie and White (1980) and Guckert *et al.* (1986). Analysis of PLFA has been demonstrated to provide reproducible, quantitative and qualitative information of the total biomass, community structure, and nutritional status of subsurface microorganisms (Nalkwill *et al.* 1988).

Microcosm studies

The microcosms consisted of 20 g (dw) sediment plus site water, with or without amendments, in sterile 60 ml. hypovials. After the addition of sediment, each hypovial was filled to capacity with amended or unamended site water, sealed with a sterile, teflon-lined Tuf-Bond septum (Pierce) and aluminium crimp seal. Amendments included: PCE only, PCE plus methanol, PCE plus acetate, or PCE plus acetate and methanol. The amendments were added to site water at concentrations of 5 mg/l, for PCE and 50 mg/l, for acetate or methanol; when both primary substrates were present, they were added at a concentration of 25 mg/l, each. Control microcosms were similarly constructed as treatment microcosms and consisted of: PCE, H₂O controls containing only PCE in sterile, distilled water; autoclaved/poisoned controls containing PCE and dissolved oxygen controls containing PCE, sediment, and site water.

Upon removal from the glovebox, the completed microcosms were inverted and placed into argon-filled paint cans equipped with GasPak Plus H₂/CO₂ generator envelopes (BBL). The cans were sealed and incubated at 10°C. Periodically a set of microcosms was removed from the incubator, warmed to room temperature, and sacrificed for analysis. Each treatment sample point is the mean result from three replicate microcosms.

RESULTS

Field. The geologic cross section (Figure 2) along transect A-A' (Figure 1) intersects the area of PCE contamination and parallels the NE to SW direction of groundwater flow. The geology is composed of clay till (upper clay) underlain in order by a sand silt unit (upper sand/silt), clay unit (lower clay), layered sand/silt unit (lower sand/silt), and bedrock. The upper clay unit is weathered, and fractured and contains numerous sand and silt lenses and stringers of uncertain lateral extent.

Dissolved chlorinated organics have been detected in groundwater samples collected from monitoring wells installed in the upper sand/silt and upper clay units. Free-phase PCE was obtained from the monitoring well TEL-3 installed in the upper clay. The lower clay unit is very stiff and appears to be a competent aquitard. The lower sand/silt unit appeared to be unconfined. The hydraulic conductivity of the upper clay unit was estimated by grain size analysis to be in the range of 10^{-8} to 10^{-9} cm/s. By contrast, the lower sand/silt layer had hydraulic conductivities of 10^{-4} to 10^{-5} cm/s in the northern portion of this unit, and 10^{-4} cm/s in the central area as determined by response tests. Differences in the hydraulic conductivities affect the ratio of vertical to lateral groundwater flow and velocity (Figure 2). Generally, groundwater flow was from NE to SE with a horizontal velocity in the central area of 0.02 to 2 m/y.

Monitoring wells were located within the upper sand/silt and upper clay units at approximate depths of 3 and 7 mbs. The geochemical results presented focus on samples collected at 7 mbs monitoring wells because there were more sampling points and greater hydraulic conductivity (and thus groundwater velocity) at this depth. The distribution of PCE, TCE, cPCE, and VC (Figure 3a, b, c, d, respectively) show similar lateral extent. cPCE was found to be the predominant isomer of PCE. Groundwater samples from ISRP 4-1 had detectable concentrations of cPCE (Figure 3c), indicating it has migrated further down-gradient. The additional lateral extent of cPCE, as compared to PCE and TCE, could be attributed to differences in the log K_{ow} of these compounds. The log K_{ow} of PCE and TCE are 2.6 and 2.5, respectively, whereas, the log K_{ow} of cPCE is 1.9. The log K_{ow} of VC (0.9) is the lowest of the chlorinated ethenes and thus would be expected to have a greater lateral distribution. Since VC did not have a greater lateral distribution, it is possible that VC is undergoing an additional transformation step.

We suspect that free-product PCE had migrated along the spur line gravel pack and found at least two points of entry into the aquifer: one centred on PW-1 and TEL-24 and the other on TEL-1 (Figure 3a). Further, distinct biological activities appeared to be associated with each centre of contamination. The samples collected from PW-1 had a higher cPCE concentration (5.540 mg/l) than the concentration of PCE or TCE (0.440 mg/l and 1.165 mg/l, respectively), whereas samples from TEL-1 had similar concentrations of cPCE and PCE (5.813 and 4.376 mg/l, respectively) and a lower TCE concentration (1.654 mg/l). The samples from ISRP 5-1 exhibited a trend similar to that of PW-1, with an elevated cPCE concentration (76.480 mg/l) as compared to either PCE and TCE, which were not detected (< 0.0001 mg/l). The concentration of VC in groundwater samples from ISRP 5-1 (9.712 mg/l) and PW-1 (1.369 mg/l) were higher than in groundwater samples from TEL-1 (0.228 mg/l).

The groundwater sample from TEL-1 had a lower ethene concentration (0.020 mg/l) compared to ethene concentrations (Figure 4a) in samples from ISRP 5-1, ISRP 4-1, and PW-1 (0.423 mg/l, 0.152 mg/l, and 0.278 mg/l, respectively). The high ethene concentration in samples from ISRP 4-1, coupled with the lack of other chlorinated ethenes, indicates that ethene has migrated from the source area near ISRP 5-1 and PW-1 (Figure 4a). Ethane was detected only in association with ethene, but at very low concentrations (ISRP 5-1, 0.010 mg/l; PW-1, 0.010 mg/l). Methanol was found at this stratigraphic level (Figure 4b).

only in groundwater samples from PW1 (810 mg/L) and ISRP 5-1 (5 mg/L), but was also detected in groundwater samples taken from wells located in higher stratigraphic units in the same area (CEL-10, 498 mg/L; ISRP 5-2, 40 mg/L; and ISRP 5-3, 1770 mg/L). Methanol was not detected in groundwater samples taken in the vicinity of CEL-1. Acetate, although more widely distributed than methanol, still appears to originate from around PW-1 and ISRP 5-1 (Figure 4c). It was detected in groundwater samples taken from ISRP 5-1 (430 mg/L) and PW1 (715 mg/L), but not CEL-1. Methane (data not shown) was detected throughout the site and did not appear to be correlated with previously presented parameters. Sulphate concentrations generally decrease in the central area of chlorinated organic contamination at the 3 mbgs level plan map (data not shown). At 7 mbgs sulphate concentrations are low in samples from PW-1 (< 10 mg/L), but are significantly greater in samples from ISRP 5-1 (230 mg/L). Chloride concentrations were elevated in groundwater samples taken from monitoring wells located in the contaminated zones. Elevated chloride levels in groundwater samples could result from dechlorination of PCE and its daughter products. The chloride plume overlaps the chlorinated organic contours and is distributed further down gradient (Figure 4d). The highest chloride concentrations were observed in groundwater samples taken from PW1 and ISRP 5-1 (360 and 380 mg/L, respectively). Chloride concentrations outside the contaminated area were typically in the 10 mg/L range.

Laboratory

Enumeration of microbial population. Viable sulphate-reducing and methanogenic populations were detected in the collected subsurface sediment based upon both culturing and phospholipid analysis. Methanogens were estimated to be less than 40 MPN per gdw with the various substrates (acetate, 4 MPN; methanol, 21 MPN; H_2 - CO_2 , 39 MPN). Sulphate reducers are present at approximately 1000 to 3000 MPN per gdw soil (acetate, 1400 MPN; lactate 3600 MPN).

PLFA analysis (Table 1) of the core material indicated that the microbial community is sparse (1 to 3×10^3 bacteria/gdw based on 10 pmole of PLFA = 10^3 bacteria), anaerobic as indicated by the dominance of anaerobic desaturase phospholipids (monoenoic w7,16 and 18's), predominantly Gram negative (ratio of PLFA to LPS lipid A hydroxy fatty acids), and stressed (cy17:0). Evidence of sulphate-reducing bacteria (SRB) was the presence of short-chain saturated PLFA. The detection of i17:1 and i17:0 PLFA were indicative of *Desulfovibrio*, and 10me16:0 (and no detection of 10Me18:0) PLFA indicate the presence of *Desulfobacter*. The biomass of SRB as estimated by pmole of SRB-PLFA (assuming 10 pmole is approximately 10^3 bacterial cells) was equivalent to SRB estimates by MPN. Isoprenoid ethers, which would indicate methanogens, were not detected at this low density of cells. These data suggest that neither sulphate reducers nor methanogens comprise a significant proportion of the microbial biomass in the sediment.

Microcosm studies. Purging site groundwater to remove residual halocarbons was not entirely successful. Table 2 lists the halocarbon content of the 5 treatment flasks of site groundwater used to prepare the various microcosm treatments. All but the unamended water received 5 mg/L added PCE, but no other halogenated compound. However, residual halocarbon, principally cDCE and excess PCE, remained. Unamended water, for example, was purged for approximately 24 h, but contained 24 mg/L cDCE. Low levels of VC were detected in the purged groundwater containing cDCE. Since VC is a highly volatile compound, we can only explain its presence as resulting from abiotic reactions of residual halocarbons with iron complexes (Vogel *et al.* 1987) or microbial activity may have occurred in the unsterilized groundwater after sampling but prior to analysis. The difference between the halocarbon concentrations in the treatment flasks (Table 2), and day zero of the microcosms (Table 3) is attributed to volatilization from the flask during dispensing of the amended groundwater. However, the concentration of PCE delivered to the microcosms appears to be constant over the microcosm construction period. This is evident in the sterile PCE- H_2O and PCE-soil control microcosms (Figure 5). The construction time difference between the day 0 and day 145 microcosms was approximately one hour, however, the concentration of PCE in these microcosms is relatively stable over the 145 day incubation period. The aquifer sediments did not significantly contribute to the day 0 halocarbon concentrations in the microcosms because sediment samples collected from the central area did not have detectable concentrations of sorbed halocarbons (data not shown).

Microbiologically active microcosms were successfully constructed from the collected subsurface material, as methane evolution and changes in the halocarbon species and concentrations were evident in active microcosm treatments, but not in the PCE- H_2O or the autoclaved/poisoned sterile controls (Figure 5). The decrease in PCE concentration in the autoclaved/poisoned controls may reflect irreversible sorption of PCE to the sediment.

Figure 6 demonstrates biologically mediated transformation of PCE in microcosms amended by PCE plus acetate/methanol. The same general transformation pattern was observed in all active (i.e., nonsterile) microcosms (Table 3). Over time, PCE was dechlorinated to TCE, then cDCE, VC, and finally, in most instances, to ethene. The transformation pattern appears strictly sequential. Small amounts of tDCE and 1,1-DCE were detected in some active microcosms up to day 46, but these were minor in comparison to the cDCE levels encountered. The prevalence of cDCE is in agreement with others (Parsons & Lage, 1985; Scholz-Muramatsu *et al.* 1990). In microcosms that contained initial concentrations of cDCE, its concentration remained unchanged while PCE decreased and TCE increased in concentration. Only when TCE was being depleted did the cDCE concentration rise (Table 3). This pattern held for each successive dechlorination daughter product. The rate of product formation and transformation also slowed as the less chlorinated products appeared.

Microcosms amended with PCE plus methanol and acetate showed more evolved ethene (0.5 mg/L at day 145) than any other amendment (Table 3). By day 145 VC was the sole halogenated compound remaining and was decreasing in concentration. Dechlorination was slowest in the PCE plus methanol treatment with vinyl chloride concentrations still increasing by day 145, whereas in all other treatments (except unamended), VC concentrations were decreasing (Table 3). The unamended treatment was probably exceptional in this regard because of the very large cDCE concentration in those microcosms. It is also notable that methane evolution was delayed in the PCE plus methanol treatment (Table 3) and no ethene was ever detected. This is in contrast to other treatments, in which ethene was present and increasing in concentration from day 83 to day 145. Indeed, in unamended microcosms ethene was detected (at the minimum detection levels) as early as day 12. Further reduction of ethene to ethane was not observed in any of the microcosms (data not shown).

Dechlorination and methane production in both unamended and amended microcosms with only PCE (Table 3) indicate that an electron donor was available in the microcosms without additional methanol or acetate. Chemical analysis of groundwater samples from ISRP 5-1 (obtained near the area from which the core material used in this study was taken) confirmed that both methanol and acetate were present in the area at concentrations from 5 to more than 810 mg/L.

Although methane was evolved in all active microcosms, no correlation between methane production and dechlorination was perceived. The methane concentration reached 41 mg/L in PCE plus acetate amended microcosms at day 145, but dechlorination was more complete in PCE plus acetate and methanol amended microcosms where methane concentration reached 6 mg/L. Dechlorination (and methane evolution) were relatively delayed in PCE plus methanol amended microcosms, but methane concentrations in that treatment were 11 mg/L at day 145.

DISCUSSION

Chlorinated aliphatics have been demonstrated to undergo anaerobic dechlorination in continuous-flow fixed-film reactors (Bouwer & McCarty, 1983; Bouwer & Wright, 1988; Vogel & McCarty, 1985, 1987) in soil (Kloepfer *et al.* 1985), sediment (Barrio-Lage *et al.* 1986, 1987; Parsons *et al.* 1985; Parsons & Lage, 1985), and aquifer microcosms (Parsons *et al.* 1984; Wilson *et al.* 1983, 1986). Although few studies have been conducted on the biotransformation of PCE in the field, many sites contaminated with PCE also have concentrations of dechlorination products in sampled groundwater and aquifer solids. Unfortunately, VC tends to persist in anaerobic environments and is more toxic than the parent compounds.

For anaerobic bioremediation to be a useful remedial method, PCE and TCE must be completely dechlorinated to nonchlorinated products. Few studies have shown the complete dechlorination of chlorinated alkenes. Vogel & McCarty (1985, 1987) have shown the conversion of PCE and VC to CO_2 . Freedman and Gossett (1989) studied the conversion of $[^{14}\text{C}]\text{PCE}$ to $[^{14}\text{C}]\text{ethene}$. Their experiments showed insignificant amounts of $[^{14}\text{C}]\text{CO}_2$ and $[^{14}\text{C}]\text{CH}_4$. They also observed that the rate limiting step was the conversion of VC to ethene, and that the presence of an electron donor was necessary. Methanol was the most effective electron donor. They found that 2-bromoethanesulfonate inhibited the conversion of PCE to ethene, which suggests that methanogens are playing a role in the conversion.

If the dechlorination products of PCE were persisting at our study site then greater downgradient transport of these products would be expected to occur. This is because of two reasons. First, the $\log K_{ow}$ value decreases with the removal of a chlorine atom. This results in the dechlorinated products having higher mobility than their more chlorinated predecessor. Second, dechlorination products will form at the leading edge of its predecessor's plume. The good degree of distribution overlap of PCE, TCE, cDCE, and VC at the study site strongly indicates the dechlorination of these compounds.

The predominance of the cDCE (as opposed to the *trans*- or 1,1-DCE isomers) is in agreement with our and other laboratory biotransformation studies of PCE (Barrio-Lage *et al.* 1986; Parsons *et al.* 1984, 1985; Parsons & Lage 1985). Methanol and acetate appears to be promoting PCE biotransformation to ethene. The evidence supporting this conclusion is the strong relationship between PCE, methanol, ethene, and acetate contours originating from the PW1 and ISRP 5-1 monitoring well locations. In contrast, PCE originating from the CEL-1 does not overlap with the methanol, acetate, and ethene plumes.

Contamination of the microcosms with residual halogenated compounds created three complications in the study: (1) the initial PCE levels were not the same in all PCE-containing microcosms; (2) the total concentration of halogenated compounds in the different microcosm treatments was highly varied, although within a set of microcosms (e.g., the "PCE plus methanol" treatment) the initial halocarbon concentration and speciation was constant; and (3) the unamended microcosms, which ideally would be devoid of halogenated compounds, contained very large amounts of cDCE. These complications limit the amount of information gained from the study, but a number of useful results and conclusions were still acquired.

The presence of cDCE in unamended microcosms prevents the unequivocal conclusion that ethene production was the consequence of vinyl chloride dechlorination because we had no active microcosms devoid of ethene. Ethene was certainly a metabolic product because it was not formed in sterile controls, but ethene is synthesized naturally by some soil microorganisms (Alexander 1977). However, it is likely that ethene was the final product of the PCE transformation sequence in the sediment, as Freedman and Gossett (1989) found in their enrichment cultures. If ethene is assumed to be the daughter product of VC, then the early appearance of this nonchlorinated daughter product in unamended microcosms was probably related to the absence of PCE and TCE. In those microcosms, the reaction sequence started at cDCE, and although the bulk of the reductive activity was

directed towards VC production, some dechlorination of VC to ethene also occurred. Despite its early appearance, ethene did not accumulate in the unamended microcosms (0.06 mg/L by day 145) nearly to the extent that it did in other ethene-positive treatments (PCE only, 0.34 mg/L; PCE plus acetate, 0.11 mg/L; PCE plus acetate and methanol, 0.51 mg/L; all after day 145), again suggesting that throughout the entire period of monitoring, most dechlorinating activity in the unamended microcosms was directed toward the large cDCE mass. This evidence, and the observation of strict sequential dechlorination of the halogenated compounds in the microcosms, indicate that reduction order, although biologically driven, was dictated by the reduction potential of the halocarbon parent-daughter product pair in question.

The microcosm experiments corroborate field evidence that PCE dechlorination is biologically-driven, and ethene is probably the final, nonchlorinated product of the sequence. Although methane was produced in all active microcosms, there was no correlation between methane evolution and dechlorination. The treatments could be ranked as follows, in terms of methane production: PCE plus acetate > PCE plus methanol > PCE only > PCE plus methanol and acetate > unamended. However, if ranked in terms of dechlorination over 145 days (indicated by final ethene concentration: initial halocarbon concentration), the ranking was quite different: PCE plus methanol and acetate > PCE only > PCE plus acetate > unamended > PCE plus methanol.

The mechanism or the microorganisms responsible for dechlorination of PCE to ethene is not clear. Few studies have examined which anaerobic microorganisms are responsible for reductive dechlorination. Methanogenic enrichment cultures (utilizing acetate) have been shown to dechlorinate aliphatics (Bouwer & McCarty 1983). Galli and McCarty (1989) have shown that a *Clostridium* sp. was able to biotransform 1,1,1-trichloroethane forming volatile acids (e.g., acetate). Egli *et al.* (1987) have demonstrated that chlorinated ethanes can be dechlorinated by *Desulfobacter autotrophicus*. Bagley and Gossett (1990) found that PCE was dechlorinated to TCE and cDCE in sulphate-reducing enrichment cultures, but that this activity decreased over time. In contrast Fathepure *et al.* (1987) found that *Desulfovibrio desulfuricans* and two species of *Clostridium* did not produce significant quantities of TCE from PCE. The authors also tested the ability of four acetate-utilizing methanogens to dechlorinate PCE. *Methanosarcina acetivorans* and *Methanohalobium* did not produce significant quantities of TCE from PCE, but the dechlorination rate of *Methanosarcina* sp. and *M. mazei* cultures paralleled methane production, and maximum yields of TCE were obtained with methanol as opposed to acetate.

In our study, the lack of a relationship between methane production and dechlorination, and the marked differences in microcosm behaviour depending upon treatment (for example, the methanol- versus acetate-amendments) suggest that: (a) activity of methanogenic bacteria was not necessarily mediating the observed dechlorination reactions; and (b) the provision of the different electron donors enriched different segments of the microbial population native to the subsurface sediment.

These points and the low population densities of methanogens and SRB in the subsurface sediments suggest that other anaerobes may be involved in the dechlorination reactions observed *in situ*. The detection of acetate in groundwater samples containing concentrations of methanol hints that acetogens may play an important role in the dechlorination of PCE to ethene. We hypothesize that methanol is serving as a substrate for acetogenic microorganisms that may be responsible for dechlorination of PCE at the study site.

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Table 1: PFLA Core Sediment Analysis¹

PFLA	Sample Location in Core	
	Top	Bottom
12:0	1.25	0.58
i14:0	0.05	0.54
14:0	5.81	3.75
i15:0	5.87	5.33
a15:0	2.76	2.46
15:0	3.54	3.32
i16:0	0.68	0.40
16:1w7c	16.21	26.16
16:1w5c	1.05	1.72
16:0	30.73	18.26
i17:1	0.00	0.79
10me16:0	0.88	0.96
i17:0	0.31	0.44
a17:0	0.08	0.38
cy17:0	9.01	15.49
17:0	0.21	0.83
18:3w6	1.31	0.00
18:2w6	0.00	0.00
i18:3w3	0.00	0.00
18:1w9c	7.47	9.21
18:1w7c	5.98	2.41
18:0	6.79	6.23
10me18:0	0.00	0.00
br19:1	0.00	0.75
cy19:0	0.00	0.00
20:1w9c	0.00	0.00
Total	100.00	100.00
pmole/gdws	29.9	11.7
<u>LPS Lipid-A OH-FA Analysis</u>		
3OH12:0	0.00	0.00
2OH12:0	100.00	0.00
2OH14:0	0.00	0.00
Total	100.00	100.00
pmole/gdws	0.13	0.00

¹Nomenclature: Total number of carbon atoms; number of double bonds with position of double bond closest to the ω end with geometry 'c' for *cis*, 't' for *trans*; 'i', 'a', and 'br' refer to iso, anteiso, and methyl-branching, respectively. Methy-branching from the Δ end is indicated by its position followed by Me and the total number of carbon atoms; Cyclopropyl fatty acids are designated as 'cy'.

Table 2: Halocarbon concentrations in purged site water after addition of PCE and substrate amendments prior to distribution to microcosms (mg/L)

Amendment	PCE	TCE	cDCE	tDCE	1,1-DCE	VC
unamended	0.36	0	24.14	0	0	0.26
PCE	2.53	0.08	3.47	0	0	0.08
PCE + methanol	7.95	0	9.46	0.002	0.001	0.42
PCE + acetate	13.92	0	0	0	0	0
PCE + methanol/acetate	11.87	0	0.67	0	0	0.003

PCE added: 5 mg/L (3.1 μ L per L), except to unamended

TABLE 3: Biotransformation Products (mg/L) of PCE by Treatment (Standard Deviation)

Treatment	Day	PCE	TCE	cDCE	VC	Ethene	Methane
PCE	0	0.372 (0.104)	0.145 (0.077)	2.350 (0.272)	0.034 (0.003)	0 (0)	0.027 (0.005)
	12	0.041 (0.059)	0.339 (0.052)	2.122 (0.321)	0.185 (0.027)	0 (0)	0.190 (0.027)
	25	0.004 (0.002)	0.029 (0.004)	2.436 (0.479)	0.279 (0.039)	0 (0)	0.491 (0.017)
	46	0.006 (0.011)	0.002 (0.004)	1.469 (0.144)	1.167 (0.071)	0 (0)	0.800 (0.123)
	83	0 (0)	0 (0)	0 (0)	1.689 (0.057)	0.092 (0.005)	4.258 (0.715)
	145	n.d.	0 (0)	0 (0)	7.290 (0.321)	0.061 (0.017)	2.624 (0.543)
PCE + Methanol	0	1.965 (0.800)	0.004 (0.006)	5.116 (0.337)	0.021 (0.007)	0 (0)	0.005 (0.005)
	12	1.019 (1.015)	0.265 (0.277)	6.015 (0.925)	0.060 (0.001)	0 (0)	0.010 (0.003)
	25	0.050 (0.006)	0.012 (0.003)	5.845 (0.954)	0.060 (0.011)	0 (0)	0.094 (0.045)
	46	0.040 (0.015)	0.010 (0.010)	4.772 (0.229)	0.177 (0.22)	0 (0)	0.185 (0.123)
	83	0 (0)	0 (0)	4.380 (0.832)	1.074 (0.033)	0 (0)	1.751 (0.519)
	145	n.d.	0 (0)	1.250 (0.581)	2.434 (0.147)	0 (0)	11.003 (1.708)
PCE + Acetate	0	0.672 (0.076)	0.003 (0.003)	0.676 (0.283)	0.002 (0.003)	0 (0)	0.008 (0.001)
	12	0.076 (0.025)	0.237 (0.037)	0.650 (0.069)	0.001 (0.002)	0 (0)	0.063 (0.005)
	25	0.024 (0.004)	0.023 (0.006)	1.095 (0.156)	0.031 (0.005)	0 (0)	0.756 (0.050)
	46	0.020 (0.015)	0.007 (0.002)	0.863 (0.058)	0.201 (0.036)	0 (0)	3.145 (0.190)
	83	0 (0)	0 (0)	0 (0)	1.055 (0.038)	0.035 (0.006)	23.818 (7.919)
	145	n.d.	0 (0)	0 (0)	0.813 (0.051)	0.109 (0.025)	40.916 (8.633)
PCE + Methanol and Acetate	0	1.641 (1.054)	0.085 (0.057)	0.627 (0.014)	0.007 (0.005)	0 (0)	0.008 (0.001)
	12	0.012 (0.002)	0.186 (0.026)	1.752 (0.162)	0.098 (0.005)	0 (0)	0.076 (0.014)
	25	0.004 (0.001)	0.013 (0.002)	1.685 (0.040)	0.229 (0.003)	0 (0)	0.655 (0.067)
	46	0 (0)	0.002 (0.004)	0.559 (0.073)	1.093 (0.026)	0 (0)	1.617 (0.101)
	83	0 (0)	0 (0)	0 (0)	1.188 (0.038)	0.238 (0.133)	4.074 (1.983)
	145	n.d.	0 (0)	0 (0)	0.175 (0.153)	0.512 (0.082)	6.033 (0.187)

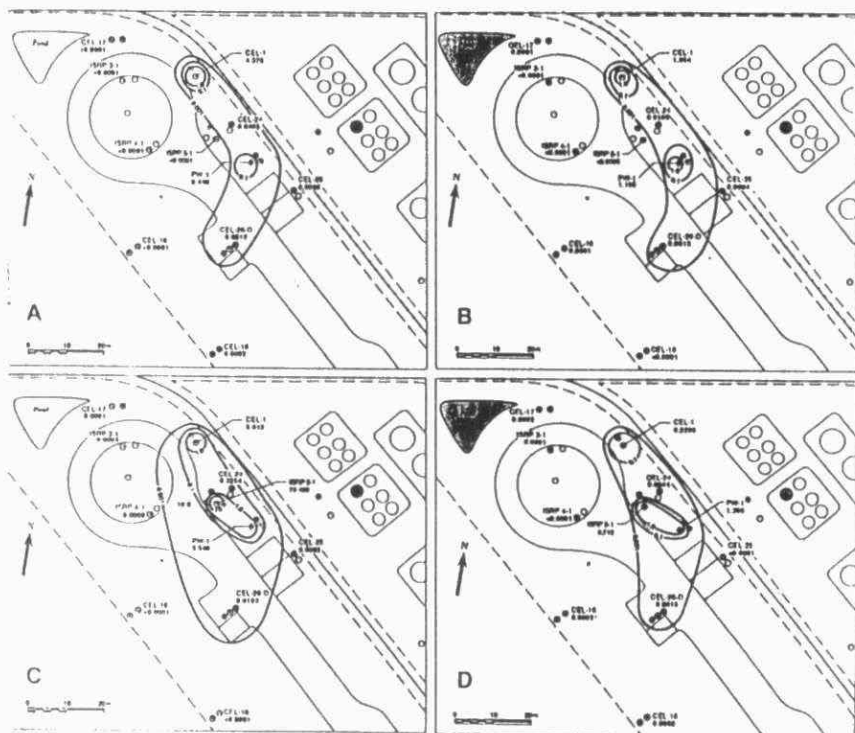


Figure 3 Contours of (A) PCE (mg/L), (B) TCE (mg/L), (C) cDCE (mg/L), and (D) VC (mg/L) in the bottom of the sand/silt at a depth of approximately 7 m bgs.

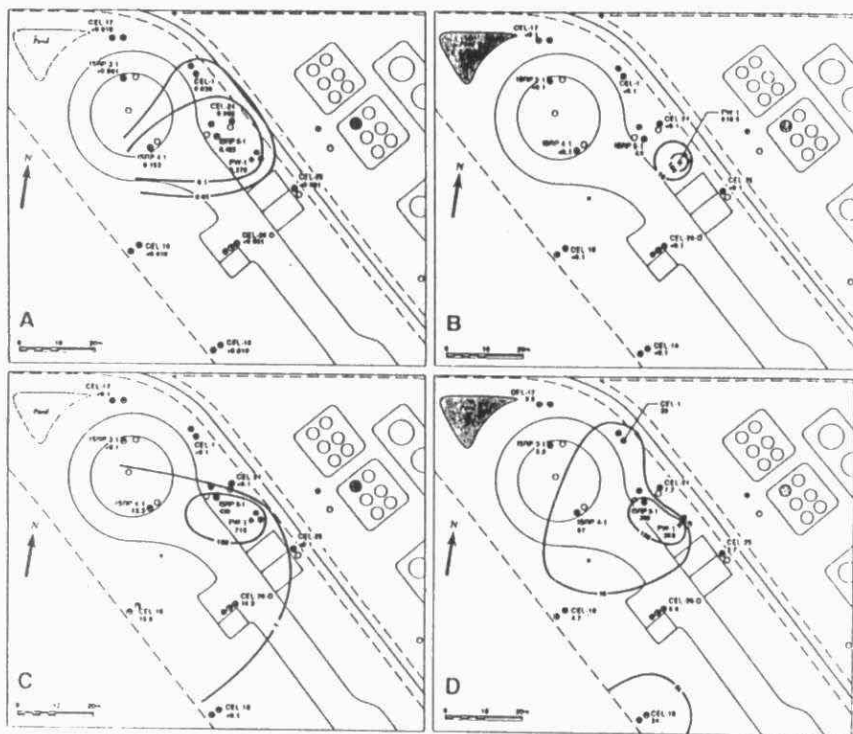
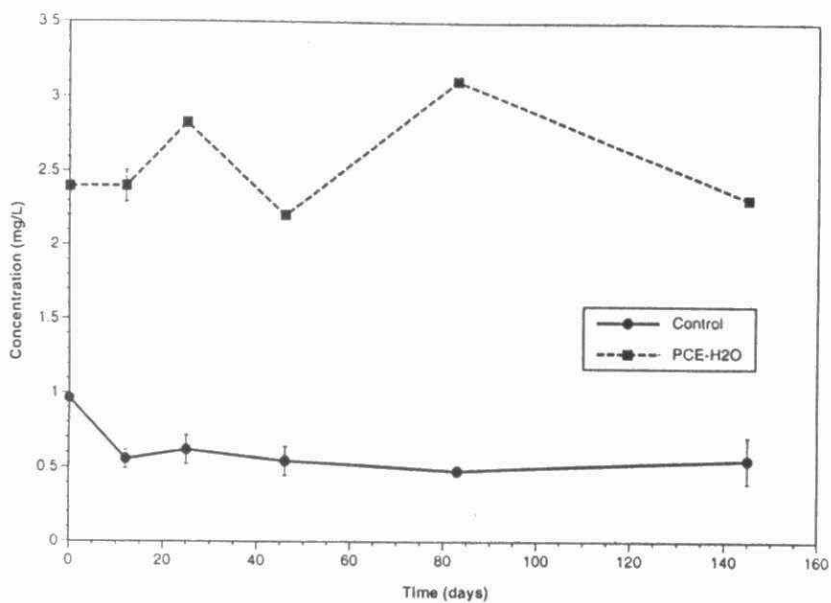


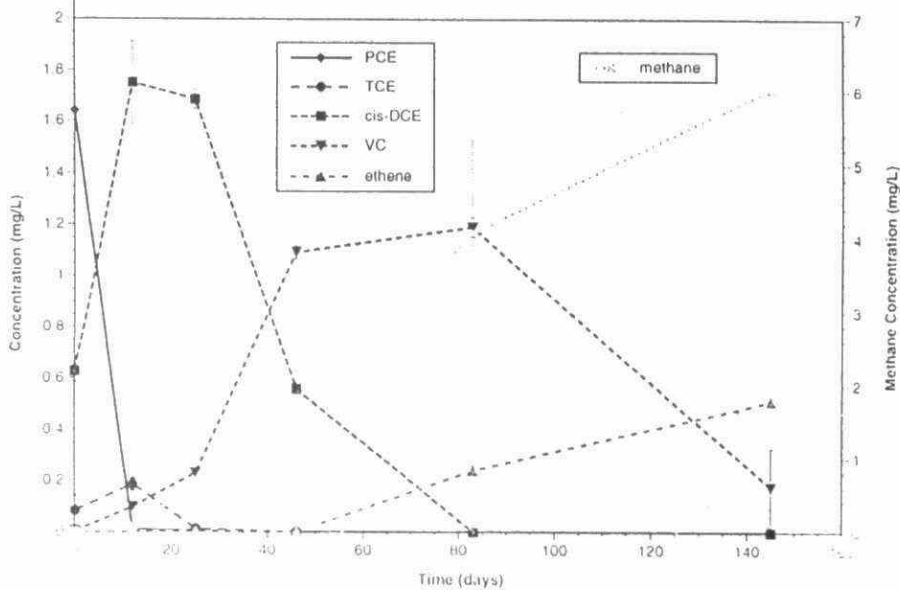
Figure 4 Contours of (A) ethene (mg/L), (B) methanol (mg/L), (C) acetic acid (mg/L), and (D) chloride (mg/L) in the bottom of the sand/silt at a depth of approximately 7 m bgs

Figure 5: PCE Controls*



* Other dechlorination products not detected

Figure 6: PCE + methanol + acetate amendment

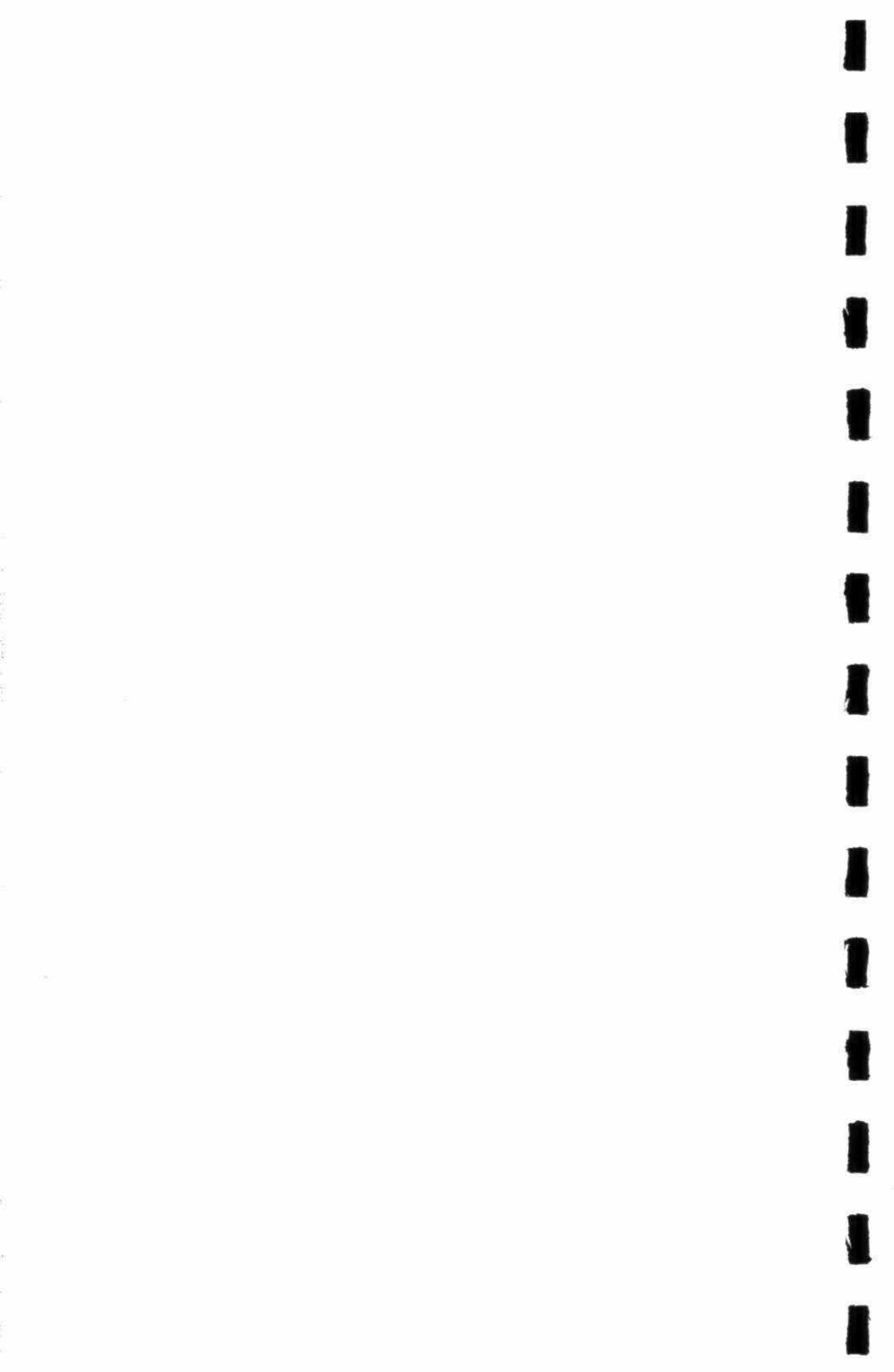


VOLUME II

SESSION F

PREVENTION, TREATMENT AND REMEDIATION

POSTER PRESENTATIONS



CHARACTERIZATION AND BIOTECHNICAL USES OF THE EXTRACELLULAR EMULSIFYING AGENT PRODUCED BY *PSEUDOMONAS AERUGINOSA*

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Biosurfactants are compounds produced on microbial cell surfaces or excreted extracellularly, which contain both hydrophilic and hydrophobic portions. Due to their amphipathic nature, surfactants aggregate at an interface, causing a reduction in surface and interfacial tensions. Since biosurfactants and bioemulsifiers both exhibit emulsification properties, bioemulsifiers are often categorized with biosurfactants, although emulsifiers may not lower surface tension.

Petroleum refineries produce large amounts of oily wastes, such as complex hydrocarbons. These hydrocarbons and some halogenated compounds are serious environmental pollutants, which may cause public health and ecological concerns due to their persistence and toxicity to biological systems. Land farming is an acceptable method for the disposal of oily sludge (Berwick and Stafford, 1985; Shailubhai, 1986). This technique involves spreading oily sludge onto soil, and providing optimal conditions to allow mineralization of hydrocarbons by soil microorganisms.

Hydrophobic compounds bind to soil particles, and are difficult to remove or degrade. Efficient degradation requires that hydrocarbons are solubilized before being degraded by microbial cells (Stucki and Alexander, 1987; Thomas *et al.*, 1986). For some hydrophobic compounds, the rate of mineralization is governed by desorption of hydrocarbons from soil (Mihelcic and Luthy, 1988a and 1988b). It is expected that surfactants can increase the surface area of hydrophobic materials, thereby increasing the water solubility. The addition of biosurfactants to the soil may enhance hydrocarbon dispersion and increase microbial degradation (Van Dyke *et al.* 1991).

Many microorganisms are able to produce surface active substances. We have been studying the characteristics of a biosurfactant produced by *Pseudomonas aeruginosa* UG2. Strain UG2 was isolated initially in our laboratory from oil contaminated soil (MacElwee *et al.*, 1990). Other researchers have shown that *P. aeruginosa* is able to produce rhamnolipid surfactants of varying structure. Jarvis and Johnson (1949), and Edward and Hayashi (1965) determined the rhamnolipid produced by *P. aeruginosa* is composed of 2 units of L-rhamnose and 2 units of β -hydroxydecanoic acid linked to the C1 position. Hirayama and Kato (1982) and Parra *et al.* (1989) determined that their *P. aeruginosa* strains produced 2 types of rhamnolipid. The first type contained 2 units each of L-rhamnose and β -hydroxydecanoic acid, while the second was similar except that it contained only one unit of L-rhamnose. *Pseudomonas* sp. DSM 2874 (Syldatk *et al.*, 1985) was found to produce 4 different types of rhamnolipids, containing either one or two units each of L-rhamnose and β -hydroxydecanoic acid. Yamaguchi *et al.* (1976) isolated a *Pseudomonas* strain that produced 2 rhamnolipids similar to that of Parra *et al.* (1989), except both glycolipids also contain 2-decanoic acid at the C2 position.

We have carried out some structural characterization of the partially purified *P. aeruginosa* UG2 biosurfactant. Analysis by HPLC and GC showed that the UG2 biosurfactant does contain L-rhamnose as the only sugar. Analysis by GC-MS indicated the presence of β -hydroxydecanoic acid and 2-decanoic acid. Therefore, we propose the structure of the *P. aeruginosa* UG2 rhamnolipid biosurfactant may be similar to those reported by Yamaguchi *et al.* (1976). Currently NMR analysis of UG2 rhamnolipids is being performed, and should provide further information on the structural arrangement of these components.

The biosurfactant produced by *P. aeruginosa* UG2 was previously found to solubilize hexachlorobiphenyl significantly in soil slurries Berg *et al.* (1990). We carried out additional partitioning studies to further investigate the ability of the UG2 biosurfactant to solubilize other hydrophobic contaminants. These studies involved spiking small amounts of soil with ^{14}C -labelled compounds, and washing the soil with surfactant solutions. The amount of ^{14}C -compound that was removed from soil into the aqueous phase was measured by scintillation counting.

In the first experiment, several hydrophobic compounds were tested in soil that contains a high amount of organic matter. The compounds tested were pyridine, naphthalene, anthracene, phenanthrene, fluorene, 2,5,2',5'-tetrachlorobiphenyl and 2,4,5,3',4',5'-hexachlorobiphenyl. All these compounds except pyridine are hydrophobic. As pyridine is water soluble, it served as a control. Partially purified, freeze dried UG2 biosurfactant was tested over the concentration range of 0.1 to 5 mg/ml.

Pyridine recovery was high (>80%) at all biosurfactant concentrations. In general, the % recovery of the more hydrophobic compounds in the aqueous phase was found to increase as UG2 biosurfactant concentration increased. However, the extent of recovery varied with the hydrocarbons. The recovery of naphthalene was 70% at 5 g biosurfactant/l, while that of anthracene, fluorene and phenanthrene were about 30-40%. The recovery of hexachlorobiphenyl was also high at 70%, while that tetrachlorobiphenyl was 40%.

These results are important because they show that all the tested hydrophobic compounds may be solubilized to varying extent by the UG2 biosurfactant. The basis for the variability in the degree of solubilization is not known. This variability is unlikely to be related to the compounds' aqueous solubilities since one of the more water-soluble (naphthalene) and one of the least water-soluble (hexachlorobiphenyl) hydrocarbons were recovered to about the same extent. More likely, the variability was dependent on factors such as soil type and content, the extent of binding (or adsorption) of the hydrophobic compounds to the soil matrix, the prevailing environmental conditions, or the nature and type of interactions between the biosurfactant and the hydrophobic substances. These are important questions that should be addressed in further studies.

In a second partitioning experiment, a sandy loam soil was used, which has a lower amount of organic matter. Again, pyridine recovery in the aqueous phase was greater than 80% at all biosurfactant concentrations tested. At 5 g biosurfactant/l, the recoveries of naphthalene, anthracene, fluorene and phenanthrene from the sandy soil were all approximately 10% higher than those from the silt loam soil. The % recovery was about 80% for naphthalene, and about 40-50% for anthracene, fluorene and phenanthrene. On the other hand, the recovery of hexachlorobiphenyl from the sandy soil was about 10% lower than that from the silt loam soil, while the recovery of tetrachlorobiphenyl was about 10 % higher. The % solubilization of both hexachlorobiphenyl and tetrachlorobiphenyl was very similar throughout the concentration range

of UG2 biosurfactant tested. This experiment clearly showed that soil type does have an effect on the ability of the UG2 biosurfactant to dislodge hydrophobic compounds from soils. Higher levels of organic matter may bind hydrophobic compounds more firmly, making removal more difficult.

In a third partitioning experiment, the ability to solubilize hydrocarbons in soil slurries was compared between the anionic chemical surfactant SDS and the anionic UG2 biosurfactant. Using the Conestogo silt loam soil, the % recoveries of naphthalene and anthracene were about the same for SDS and UG2 surfactants. The % recoveries of phenanthrene, fluorene, tetrachlorobiphenyl and hexachlorobiphenyl were clearly higher using the UG2 biosurfactant. The same experiment was repeated using the sandy loam soil. The % recovery of naphthalene was approximately the same using SDS and UG2 surfactant, but the recoveries of anthracene, phenanthrene and fluorene were about 10% higher using the UG2 biosurfactant than using SDS. Recoveries of hexachlorobiphenyl and tetrachlorobiphenyl using the UG2 biosurfactant were over 20% higher than using SDS. These experiments demonstrated that the UG2 biosurfactant is superior to SDS in the partitioning of hydrophobic compounds from soil into the aqueous phase.

Studies are in progress to investigate the effect of UG2 biosurfactant on microbial degradation of hydrocarbons in soil. Solubilization and degradation experiments may show that biosurfactants can be used to enhance recovery of pollutants from contaminated soil.

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**Investigation of Ontario Mixed Layer Mica-Vermiculite
as Potential Landfill Liner Material and Adsorbent
of Organic and Inorganic Pollutants**

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Clay minerals in clay soils which are commonly used as landfill liners, must have a low permeability and high filtering ability. Although all types of locally available clays are used, the most suitable type of clays possess the following characteristics: high cation exchange capacity, large specific surface area and the presence of surface functional groups (hydroxylated edges, broken chemical bonds, sites with organic or inorganic ions).

Glaciolacustrine clays which are widespread in southern Ontario often contain illite and chlorite. These clays generally have low cation exchange capacities (C.E.C.) and low specific surface areas. The more suitable Champlain Sea clays which are used in Quebec as landfill liners and substrata, have a better C.E.C. when compared to the glaciolacustrine clays (50 meq/100 g versus 20 meq/100 g). Smectite group clays and vermiculite have a specific surface area which is an order of magnitude larger than that of illite and chlorite. Vermiculite has one of the highest C.E.C.'s among the clay minerals, and would appear to be a desirable component for landfill liners.

Although commercial grade deposits of vermiculite are presently unknown in Ontario, an occurrence of mixed layer mica-vermiculite in southern Ontario has been documented by MacKinnon et al (1990). This site was chosen for study, in particular since a large stockpile of the material was present, which could be readily bulk sampled (Figure 1). The deposit occurs 13 km south of Perth, approximately 350 km from Toronto, and 80 km from Ottawa. The deposit has been previously assessed to determine the viability of using it as a source of expanded vermiculite (which is used as an insulator, an absorbent of fertilizers, etc.).

Samples of vermiculite from the Stanleyville deposit as well as the hydrothermally clay altered host rock were studied by petrography, x-ray diffraction, electron microprobe analysis and scanning electron microscopy.

In addition, selected samples were evaluated to determine their C.E.C.'s and their ability to retain heavy metals when subjected to leaching tests using landfill leachate solutions.

RESULTS

Chemical Composition and Mineralogy

Electron microprobe analysis suggests that the 'micas' at Stanleyville range in composition from serpentine to phlogopite. The low Al content of the Stanleyville 'micas' is notable when compared to commercial vermiculite deposits (Figure 2). Individual grains from Stanleyville samples show a wide variation in K/Mg and K/Al ratios, as confirmed by microprobe and SEM analysis.

XRD analysis was subsequently performed on samples collected from the open pit and trenches. Some samples were treated with KCl, NaCl, MgCl₂ or NH₄-acetate, in addition to heating and ethylene glycol treatments.

The results of this work suggest that much of the coarser material previously classed as vermiculite in Stanleyville is in fact a mixture of phlogopite and lizardite, contained within a matrix of aliettite, smectite, talc and vermiculite, with minor amounts of hydrobiotite. Aliettite, present in all samples, is a regularly interstratified mixed-layer clay mineral in which talc and saponite are the constituent layers. A number of XRD profiles illustrating the behaviour of the Stanleyville aliettite and vermiculite when subjected to various treatments are shown in Figures 2, 3 and 4.

In view of the lower than anticipated vermiculite content, the samples from Stanleyville were expected to have a low cation exchange capacity. Predicted C.E.C.'s for the samples analyzed based on XRD and mineralogical analysis ranged from 15 meq/100 g to 37 meq/100 g.

Soil Chemistry

Cation exchange and heavy metal retention tests were conducted on a variety of samples. The C.E.C.'s were determined using batch equilibrium tests and by the silver-thiourea method. Results indicated that exchangeable Na⁺ and K⁺ were low (<0.5 meq/100 g); and exchangeable Ca²⁺ and Mg²⁺ moderate to high (1 to 17 meq/100 g). Total C.E.C.'s were in the order of 7 to 19 meq/100 g. Grinding of several fractions did not produce a significant increase in C.E.C.

Heavy metal retention tests were conducted using spiked solutions from an actual landfill site. The composition of the leachate is shown in Table 1.

TABLE 1. LEACHATE CHARACTERIZATION*
(mg/L or ppm)

PHYSICAL	ORGANICS	ANIONS	CATIONS	HEAVY METALS
pH 7.06	Oil and Grease 17	NH ₄ 192	Na ⁺ 144.9	Pb ²⁺ 35.4
Conductivity (u ohm/cm) 8903		NO ₂ ⁻ , NO ₃ ⁻ <0.1	K ⁺ 74.9	Zn ²⁺ 38.3
Alkalinity 4340		Cl ⁻ 1515	Ca ²⁺ 23.48	Cr ³⁺ 28.4
		SO ₄ ²⁻ 27.1	Mg ²⁺ 21.7	Cd ²⁺ 1.2

* - unspiked

The results of the heavy metal retention studies with low heavy metal contents demonstrated that retention was high, being greater than 90% in all cases. Two of the soils with higher C.E.C. were tested using spiked leachate with high heavy metal concentrations (Pb²⁺ up to 6380 ppm, Zn²⁺ up to 2400 ppm). It was noted that the retention of the Pb²⁺ and Zn²⁺ increased with the initial cation concentration used and was maximized at about 500 ppm. At high concentrations, the % adsorbed is lower (<50%), as indicated for one example shown in Table 2. It was also noted that the maximum amount of these heavy metals retained could exceed the C.E.C. of the soil. Retention of up to 24 meq/100 g of Zn²⁺ and 36 meq/100 g of Pb²⁺ was achieved over the range of heavy metal concentrations studied. An increase in pH of the effluent solution was noted after the tests (initial pH = 1-2, final pH = 2-4).

The specific surface area of the soils are moderately high, ranging from 90 m²/g to 206 m²/g. Determinations of permeability on one sample compacted wet of optimum moisture content (14.4%) indicated an average value of 1.2 x 10⁻⁷ cm/sec.

TABLE 2. PARTIAL RESULTS OF HEAVY METAL RETENTION (HIGH CONCENTRATIONS)
(Soil No. 10, C.E.C. = 18.59 meq/100g)

Pb ²⁺				Zn ²⁺				Pb ²⁺ , Zn ²⁺ or (Pb ²⁺ +Zn ²⁺)
Influent (ppm)	Effluent (ppm)	Adsorbed (ppm)	% Adsorbed	Influent (ppm)	Effluent (ppm)	Adsorbed (ppm)	% Adsorbed	Adsorbed (meq/100g)
500	60	440	88.0	-	-	-	-	4.25
1000	320	680	68.0	-	-	-	-	6.56
3000	1750	1250	41.7	-	-	-	-	12.07
6000	3650	2150	35.8	-	-	-	-	20.75
-	-	-	-	600	232	368	61.3	11.26
-	-	-	-	1200	730	470	39.2	14.38
-	-	-	-	2400	1620	780	32.5	23.65
500	250	250	50.0	150	79	71	74.3	4.58
1000	610	390	39.0	300	182	118	39.3	7.37
2000	1050	950	47.5	600	364	236	39.3	16.39
4000	3075	925	23.1	1200	775	425	35.4	21.93
"8000" (3400)	3000	400	11.8	"2400" (2350)	1300	1050	44.7	35.98
-*	0*	-*	-*	-*	0.2*	-*	-*	-*

* - Test blank

() - Actual concentration

CONCLUSIONS

Results to date suggest the Stanleyville deposit is composed of phlogopite, lizardite, aliettite, smectite, talc, vermiculite and minor hydrobiotite. Due to the lower than expected vermiculite content, the C.E.C.'s were low; however, the total adsorbed cation (Pb^{2+} , Zn^{2+} , Na^+ , K^+ , Mg^{2+} and Ca^{2+}) retention can exceed the C.E.C. of the soil. Given the low pH values of the effluent solutions, precipitation of the heavy metals is unlikely to explain this observation. Secondly, there appears to be a strong retention of Ca^{2+} and Mg^{2+} which contributes significantly to the high C.E.C. The specific surface areas of the soils are moderately high, and selected compacted soils returned low permeability results.

Given these characteristics, as well as the ability of the minerals to create a moderately alkaline pH in the aqueous environment, the deposit at Perth may constitute a speciality resource for the remediation of certain toxic pollutants. Future investigations being considered relate to identifying the mechanism of contaminant retention, determining the organic pollutant retention capabilities, and the probability of increasing the C.E.C. of the stockpiled material via clay mixing processes.

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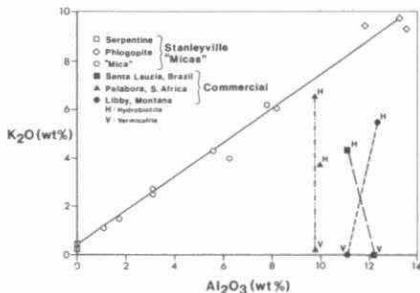
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Figure 1



Geological map of the Olympus Mines Ltd. property (after Guillet 1962).

Figure 2



Potassium versus aluminum plot for 'Mica' grains from Stanleyville and selected commercial deposits.

Figure 3 (a)

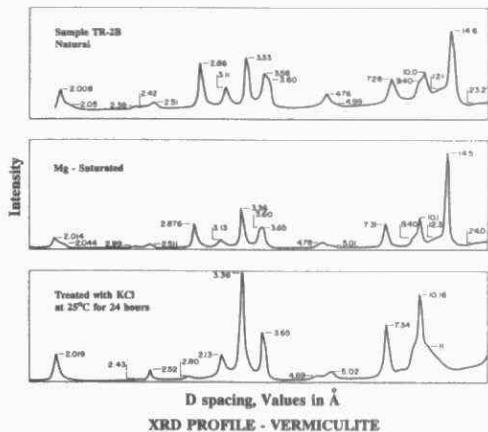


Figure 4 (a)

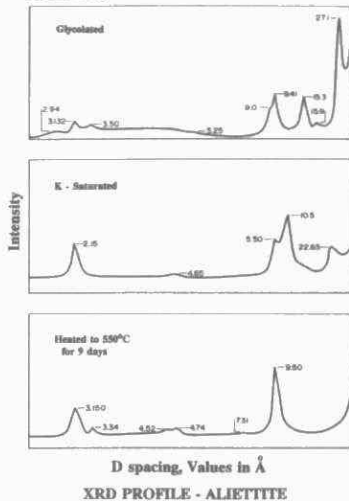


Figure 3 (b)

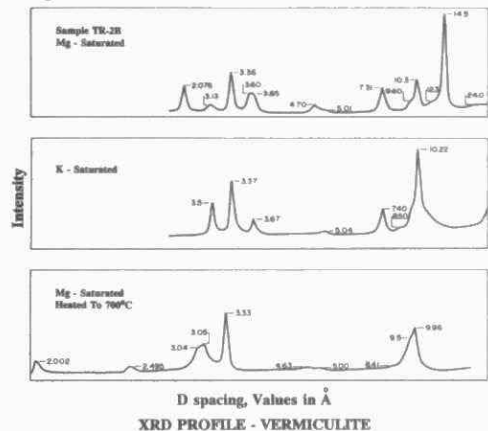


Figure 4 (b)

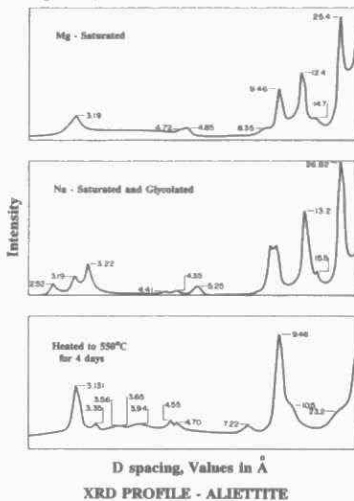


Figure 5 Heavy Metal Retention

Figure 5 (a)

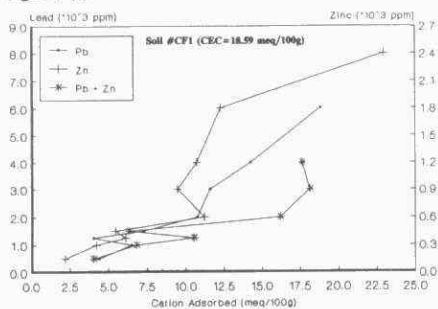


Figure 5 (d)

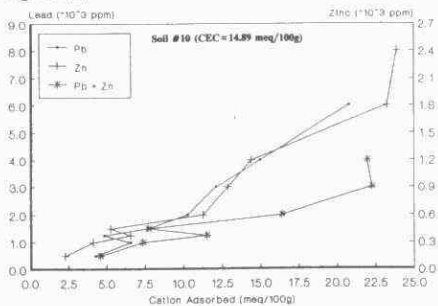


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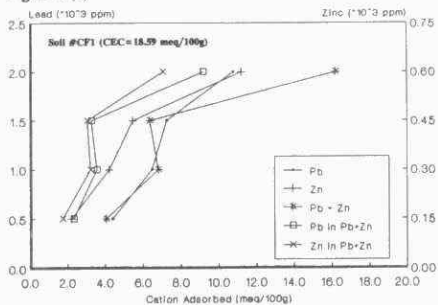


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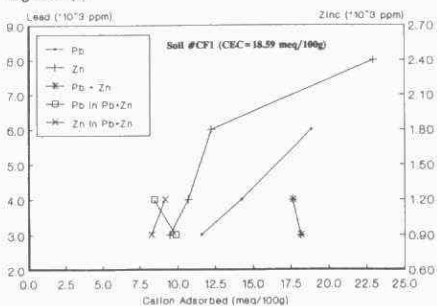


Figure 5 (c)

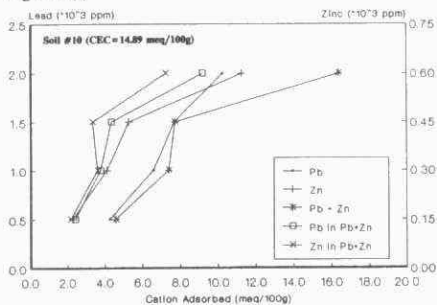
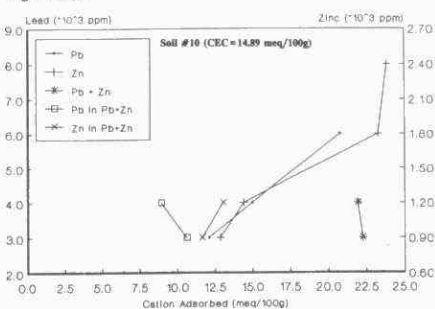


Figure 5 (f)



FLASH PHOTOLYSIS/HPLC METHOD FOR DETERMINING THE SEQUENCE OF
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NOVEMBER 1991

A new method involving the use of flash photolysis followed by rapid high performance liquid chromatography analysis is applied to the examination of the photodegradation of phenol (I) and 4-chlorophenol (II) in undegassed aqueous solution (10^{-4} - 10^{-3} M). Analysis of the solution following a single flash (direct photolysis) indicates that *p*-benzoquinone (III) is the only major photoproduct from the photolysis of either I or II. After several flashes other products appear, namely hydroquinone (IV) and 2-hydroxy-*p*-benzoquinone (V), which are known photoproducts from the direct photolysis of III. This was confirmed by a study of III subjected to a single flash. In other experiments, solutions of II containing varying amounts of H_2O_2 were subjected to flash photolysis. When the ratio of $[H_2O_2]$ to II exceeded 20:1, the product distribution changed such that 4-chlorocatechol (VI) is the major photoproduct with a smaller amount of IV and 1,2,4,-trihydroxybenzene (VII). The results will be discussed and mechanisms proposed.

INTRODUCTION

Although a number of water purification techniques are available, the oxidation of aqueous organic pollutants is an attractive method because of its high efficiency and simplicity (5,6). The oxidation products are usually low

[†] Most of the work presented in this paper has been published, is in press or has been submitted (see references 1-4).

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molecular-weight oxygenated compounds that are easily biodegradable. In the past decade or so, the potential use of the photooxidation of organic pollutants in the presence of H_2O_2 has aroused the considerable interest in the environmental community.

Phenols and chlorophenols are common pollutants found in effluents from pulp mills and in the production of fungicides and herbicides. Since previous studies have yielded a rather confusing and incomplete picture concerning the photolysis of phenols and chlorophenols in model and natural systems, we have undertaken a detailed study of the sequence of products formed in both direct photolysis and photolysis mediated by hydrogen peroxide.

To address the problems encountered in previous investigations, we have developed a method utilizing the technique of flash photolysis; however, instead of using the conventional configuration where subsequent reactions are followed by detecting the optical absorption of transients, we have employed HPLC detection to detect and identify moderately-lived intermediates. With this approach we can obtain a significant fraction of conversion with a single flash and thus avoid the possibility of photolysis of intermediates. Also by using multiple flashes, we can follow the sequence of intermediates in the reaction. This technique is limited to the detection of intermediates with lifetimes greater than ~15 min, i.e., the time required to complete an HPLC run.

We have used phenol (I) and 4-chlorophenol (II) as model compounds for testing the method, since the photoreaction of these compounds has been studied using conventional approaches (7,8), which have yielded a much more complex series of photoproducts.

RESULTS AND DISCUSSION

In the first set of experiments, undegassed solutions of I and II were subjected to 1 - 30 flashes (45 J) and analyzed within 2 min. by HPLC. After only one flash *p*-benzoquinone (III) is the only photoproduct. Its identity was confirmed by isolation and analysis by FTIR, 1H FTNMR, UV and mass spectrometry. After 5 - 10 flashes, new compounds begin to appear: hydroquinone (IV) and 2-hydroxy-*p*-benzoquinone (V), which are known photoproducts from the photolysis of III. This was confirmed by flashing solutions of III in a separate series of experiments.

After 20 - 30 flashes, traces of 1,2,4-trihydroxybenzene (VII) are observed in the case of the photolysis of I and both VII and 4-chlorocatechol (VI) in the case of the photolysis of II.

In a second set of experiments we have examined the distribution of photoproducts from the flash photolysis of II as a function of the ratio of the concentration of H_2O_2 to that of II, kept at 9.6×10^{-4} M. At concentration ratios between 0.6 and 20, the amount of III formed decreases, while the amounts of IV and VI increase significantly. This shows a clear changeover of products from those found in direct photolysis to those found in the hydrogen peroxide mediated photolysis.

It has been known for a long time that hydrogen peroxide photolyses to produce $\cdot OH$ radicals, which react with aromatic compounds by adding to the ring to produce hydroxylated products. Thus, the observation that VI is the major product observed after a single flash is quite understandable. Some $\cdot OH$ radicals also displace chlorine atoms in II since IV and V are significant products.

In a final set of experiments, the direct photolysis quantum yield was found to be 0.13 for I and 0.24 for II using a broadband technique based on the photolysis of III as an actinometer (quantum yield = 0.47).

CONCLUSIONS

1. When studied at low concentration, where bimolecular reaction steps are slow, the photochemistry of phenol and 4-chlorophenol is particularly simple, yielding *p*-benzoquinone as the major photoproduct.
2. In the case of II, as the ratio $[H_2O_2]/[II]$ increases, the photoreaction mechanism changes from direct photolysis (where III is the major direct photoproduct) to H_2O_2 mediated photolysis (where VI is the major photoproduct).

Finally, we conclude that the new technique of flash photolysis/HPLC is very effective in elucidating the detailed reaction sequence of aqueous photochemical reactions of environmental interest.

ACKNOWLEDGEMENTS

This research was supported by a Grant from the Ontario Ministry of the Environment (Project No. 487G).

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DEVELOPMENT OF A HEPATIC MICRONUCLEUS ASSAY IN FISH, C.D. Metcalfe and C.R. Williams, Environmental and Resource Studies Program, Trent University, Peterborough, Ontario K9J 7B8.

An in vivo hepatic micronucleus assay with rainbow trout is being developed to test for the genotoxic effects of aquatic contaminants. An assay protocol has been developed in which proliferation of hepatocytes in the trout liver is stimulated by a regenerative response after exposure to allyl formate (AF). Two protocols have been tested in which trout are exposed to the clastogen before or after exposure to AF. Both protocols give a positive response with the indirect-acting clastogen, diethylnitrosamine (DEN). The direct-acting clastogens, ethyl methanesulphonate and mitomycin C, have been tested using the protocol in which AF exposure occurs first, and both of these compounds gave a positive response in this assay. Tests are continuing with other genotoxic chemicals, including the PAH compound, benzo(a)pyrene.

EPR SPIN TRAPPING DETECTION OF SHORT-LIVED RADICAL INTERMEDIATES
IN THE DIRECT PHOTOLYSIS OF 4-CHLOROPHENOL IN AERATED AQUEOUS SOLUTION[†]E. Lipczynska-Kochany,[†] J. Kochany[†] and J. R. BoltonDepartment of Chemistry, The University of Western Ontario
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NOVEMBER 1991

The photolysis of 4-chlorophenol (I) in aqueous solution (pH = 6.5 to 10.5) in the presence of the spin trap 5,5-dimethylpyrroline-N-oxide (DMPO) results in the formation of at least five distinct EPR spectra. We have identified three of these as arising from the DMPO spin adducts of: the hydrogen atom [or a hydrated electron plus later protonation] (spectrum A), an aryl radical [probably the *p*-hydroxyphenyl radical] (spectrum B) and the $\cdot\text{OH}$ radical (spectrum C). Spectrum D probably arises from a decomposition product of one or more of the spin adducts, and spectrum E is that of the *p*-benzoquinone anion. By examining EPR spectra after various times of uv irradiation and determining the time course of the growth of each spectrum, we have established that spectra A and B appear immediately on initiation of the photolysis. In contrast, the growth of spectrum C is sigmoidal, indicating that it arises from a secondary product. We present a mechanism, based on the primary radicals detected, that accounts for the photoconversion of I to *p*-benzoquinone (II).

INTRODUCTION

The direct photolysis of organic pollutants (mostly aromatic) in the natural environment is stimulated by solar radiation in the range 290-400 nm. In addition, artificial photolysis, using uv lamps with a large fraction of their output below 300 nm, is gaining interest as a commercial process for the removal of these organic pollutants. Unfortunately, very little is known about

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the detailed mechanism of the direct photolysis of most aromatic organic pollutants in aqueous solution.

Chlorophenols are found as important pollutants in effluents from pulp and paper mills, as well as in the production of pesticides and herbicides. Some information is available concerning the longer-lived photoproducts. Boule *et al.* (1) found that the reaction is non-specific, giving *p*-benzoquinone, hydroquinone, biphenyls and polyphenolylic oligomers. Recently we have reexamined this photochemical reaction under more dilute conditions using a flash photolysis/HPLC technique (2) and find that *p*-benzoquinone (II) is the only major photoproduct from the direct photolysis of 4-chlorophenol (I) in aqueous solution. In spite of this knowledge, relatively little is known about the detailed mechanism leading to the known stable photoproducts.

The technique of spin trapping, with electron paramagnetic resonance (EPR) detection of free radical intermediates (3), has proven to be valuable in the study of the mechanisms of photolysis reactions. For example, it has been shown that $\cdot\text{OH}$ radicals are produced as a primary product in the photolysis of *p*-benzoquinone (II) in aqueous solution (4). We have recently used this technique to determine the rate constants for the reaction of $\cdot\text{OH}$ radicals with various chlorophenols (5a) and halobenzene derivatives (5b) in aqueous solution.

The purpose of the present work was to investigate the mechanism of the formation of II from the photolysis of I using the spin-trapping technique with EPR spectroscopy.

RESULTS AND DISCUSSION

When I is photolyzed by uv light in the EPR cavity in the presence of DMPO at pH 8.5, several EPR spectra can be detected. A computer reconstruction reveals that there are at least four distinct spectra, which we have been able to assign to the following radicals: (A) the hydrogen atom DMPO spin adduct; (B) an aryl (phenyl type) DMPO spin adduct; (C) the $\cdot\text{OH}$ radical DMPO spin adduct; (D) an unidentified radical with a single interacting nitrogen nucleus, which we suspect to be a decomposition product of one of the DMPO spin adducts.

Most of the spectral features are well reproduced by the simulated EPR, and we have found that we can fit almost all the EPR spectra under various conditions by simulations made up of varying proportions of the four base EPR

spectra. In addition, under some conditions (especially at higher pH) the EPR spectrum of the *p*-benzosemiquinone anion radical is observed.

As the uv irradiation progresses, spectrum C increases in relation to the others. This indicates that the $\cdot\text{OH}$ spin adduct is likely formed in a secondary reaction. As further confirmation we have examined the time course of the rise of the EPR signal for four field positions characteristic of each spectral species. Spectra A and B rise immediately on initiation of the uv irradiation, indicating that these probably arise from primary radicals; however, spectrum C exhibits a sigmoidal rise, indicating that it arises from a secondary radical. We propose that spectrum C arises from the generation of $\cdot\text{OH}$ radicals from the photolysis of II produced from the photolysis of I. Although spectrum D overlaps a line from spectrum A, we believe that species D also arises from a secondary photochemical process.

As a control we have carried out a series of experiments in which we have irradiated phenol (III) in the presence of DMPO under the same conditions as for the photolysis of I. We have found that the contribution from species B is much less in the photolysis of III as compared to I. We interpret these observations to indicate that the carbon-centered radical (B), produced in the photolysis of I, arises primarily from the homolytic fission of the carbon-chlorine bond of I. The fact that hydrogen atoms (or hydrated electron followed by protonation) are generated from the photolysis of both I and III indicates that they arise from the photochemistry of the phenol chromophore.

We observed previously (2) that, on bubbling with a gas stream containing progressively lower partial pressures of oxygen, the yield of II dropped off significantly, indicating that the photoreaction of I probably involves oxygen as a reactant. We find that the EPR spectrum shows a much stronger contribution from spectrum C (the $\cdot\text{OH}$ spin adduct) in aerated solutions as compared with the EPR spectrum in nitrogen bubbled solutions. This is expected if more II is formed in the aerated solution, as compared with the nitrogen-bubbled solution.

Although the overall appearance of the EPR spectra change with increasing pH, the component EPR spectra do not, except at high pH where the EPR spectrum (Spectrum E) of the *p*-benzosemiquinone anion is detected. The generation of this species is independent of the presence of DMPO.

Our observations lead us to the conclusion that the only primary free radicals produced from the photolysis of I are hydrogen atoms (or hydrated electrons followed by protonation) and an aryl (probably *p*-hydroxyphenyl) radical. The control experiments with phenol indicate that the hydrogen atom arises from the phenol entity.

Ononye *et al.* (4) have shown that the excited state of II attacks water to produce *p*-benzosemiquinone and hydroxyl radicals with a high quantum yield (~ 0.5). In the present studies, the generation of *p*-benzosemiquinone anion radicals from the photolysis of I was detected by EPR. Hence our present observation that the DMPO-OH spin adduct arises from a secondary photolysis reaction, and the detection of the *p*-benzosemiquinone anion in photolyzed solutions of I, supports the proposal that most of the $\cdot\text{OH}$ radicals are generated from the secondary photolysis of II.

CONCLUSIONS

Our spin-trapping EPR experiments have permitted a much more detailed description of the mechanism of the photolysis of I and of III on route to formation of III. Further details will be presented in the poster.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Ontario Ministry of the Environment and the Natural Sciences and Engineering Research Council, for which we give grateful thanks.

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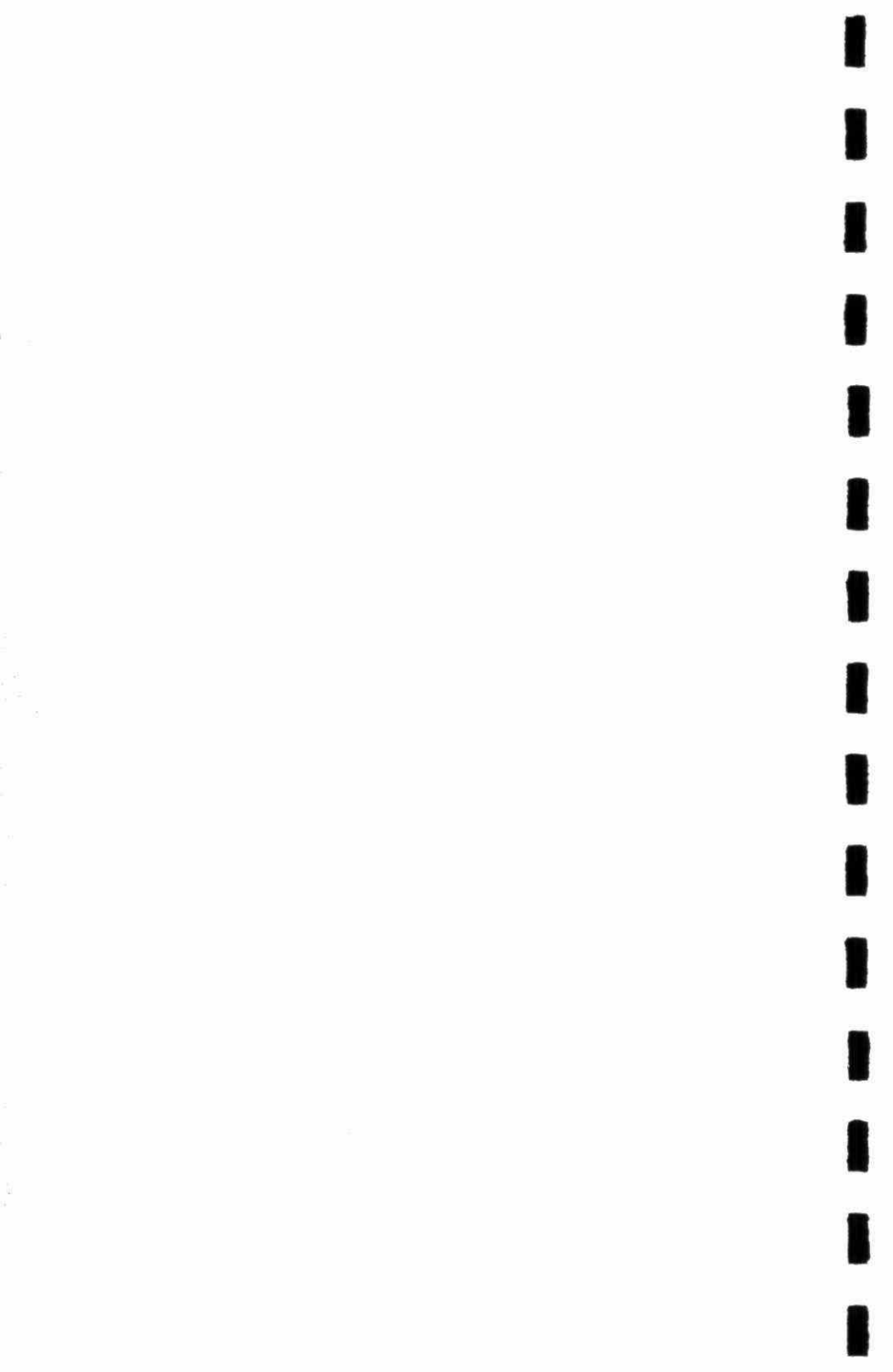
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VERBAL PRESENTATIONS



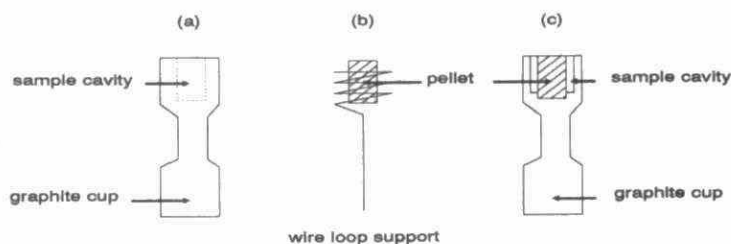
A COMPARISON OF ELECTROTHERMAL VAPORIZATION AND DIRECT SAMPLE INSERTION FOR THE ANALYSIS SOLIDS BY ICP-AES

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It is important to appreciate that most of the inhabited world is solid. While we have an active interest in water and air, we live on the land. To monitor our environment, we must then monitor the land and its products. Even the air and water exist in an equilibrium with the land. In this equilibrium, the land surface is often the major contributor of environmental contaminants. With this simplification in mind, one can appreciate that most environmental samples are solid or, if liquid, very far from being pure water. This has led us to concentrate our elemental analysis research on the development of new solid sample analysis methodologies. In our work on sample introduction methodologies we have come to appreciate the difference between academic and "real world" analytical environments. An appreciation of the real world can lead to an alteration in the way in which the analytical chemistry must be done. Our work with Direct Sample Insertion (DSI) analysis techniques for inductively coupled plasma atomic emission spectrometry (ICP-AES) started in the early nineteen eighties. The DSI technique requires the use of a sample carrying probe as illustrated in Figure 1.

The sample is placed in or on the probe, and then inserted into the plasma along the central axis. A number of apparent advantages accrue, foremost among them, 100% sample delivery. If the delivery is over a short length of time, then improved detection limits can be realized (1). In fact, we were even able to realize sub 1% relative standard deviations when the system was automated and liquid samples were inserted with a wire loop probe (2). There can also be some negative aspects to using a sample carrying probe: [1] The probe can break down and contribute to the spectrum, and [2] the sample can react with the probe. Nonetheless, the promise of 100% sample delivery is very attractive. As we will see, however, the delivery of 100% of a real sample may not be as desirable as one would originally expect. This, in turn, leads us to consider other ways in which we might achieve the desired analytical goals.

FIGURE 1



DSI Analysis of Geological Materials

Our initial work with DSI systems and liquid samples was very successful. Detection limits were improved by factors of roughly 10-100 and precision of the order of 0.5 - 1% was obtained with pure water standards. We immediately began an aggressive assault on solid samples. A study indicated that estuarine sediments were of considerable interest to the MOE as they constituted a significant fraction of the total work load in both numbers and difficulty of converting to a liquid form suitable for analysis by conventional ICP sample introductions systems.

CONVENTIONAL DSI

Our first approach involved the use of conventional probes, slightly modified d.c. arc electrodes. This work indicated that we were going to have troubles with detection limits. Unlike our convenient solid standards, dilute oxides in graphite, real geological samples were going to be a problem. Geological samples have a matrix, usually some form of Si, which is resistant to low power chemical and physical attack. The traditional ICP-AES methodology commonly involves digestion with HF for a significant length of time. The DSI-ICP-AES approach involved the use of high temperatures, approximately 2000 degrees Centigrade. Given the excellent excitation characteristics of the plasma, one might expect much higher temperatures; however, one must keep in mind that the ICP, while an excellent *spectral excitation source*, is not a particularly good *heater*. It is usually operated at about 1 kW, roughly the power of an automobile block heater, and the majority of the power goes to maintaining the plasma, rather than heating the probe. The result is that geological Si based samples usually melt to form a glass type bead which only slowly breaks down. Volatile elements emerge quickly but their signals are highly erratic due to the volcanic effect of the matrix. To avoid the formation of beads, the sample could be diluted in graphite. This led to unsatisfactory detection limits.

PELLET DSI

In an attempt to increase sample mass and yet dilute the sample, we tried a radical approach, the use of graphite pellets (3) as illustrated in Figure 1 (b). Common sense indicated that one could insert only a limited size probe into the ICP. To obtain optimal heating, we simply eliminated the graphite cup which we considered to be an unnecessary heat sink. This arrangement allowed sample masses approximately one order of magnitude larger than that which was possible with conventional probes. A secondary, but very important benefit, was the improvement in precision obtained, from 50% *rsd* to 8% *rsd*. A number of facts emerged as we studied DSI-ICP-AES. The first was that excellent detection limits, of the order of 10 ppb, could be obtained for ideal standards. Work with the MOE using ICP-MS also showed excellent results (6).

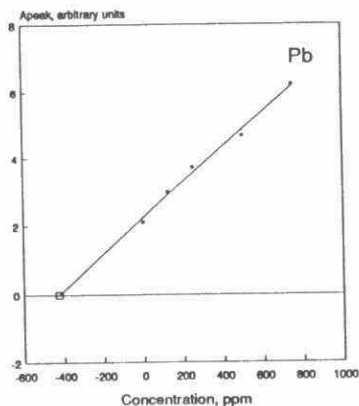
Experimentation with geological standards was extremely informative although disappointing. In short, visual observations served to illustrate the effect that the massive release of Si was having on the plasma. The gaseous Si was clearly disrupting the excitation zone and changing excitation conditions. Only the volatile elements, which emerged before the Si phase, could be analyzed.

PELLET-CUP PROBES

The final stage of the DSI system is presented in Figure 1 (c). A pellet is inserted inside a specially machined graphite cup. The cup directs all vapors upwards ensuring that the plasma is minimally perturbed and that analyte is concentrated in the viewing zone of the plasma. The analyte remains diluted in a pellet providing all the previously discussed advantages. Gases escaping from the pellet move up the space between the inner cup wall and the outer wall of pellet (4,5).

This arrangement allows the additional of chemical additives which can cause the breakdown of the matrix as well as the formation of more volatile analyte species. In our work, AgCl was found to be superior. In some cases it was found that the use of internal standards or standard additions provided superior analytical performance as indicated in Figure 2.

FIGURE 2



PELLET-ETV-ICP

Even before the DSI work above was finished, we had begun work with a pellet ETV-ICP-AES system (7). The rationale behind the pellet approach was identical to that which inspired the original pellet DSI-ICP-AES work: Dilution provides better performance and higher masses provide better detection limits and better precision.

The ETV device used is a modified Perkin-Elmer HGA-2200 graphite furnace. The modification includes the replacement of the original graphite contact rings with home-made brass ones, the construction of a brass "sample holder" and a Pyrex glass chamber, and the use of O-rings for sealing.

Pellets were made of oxides of volatile elements (As, Cd, Hg, Mn, Pb and Zn) are run first to characterize the system. The preparation of pellets includes mixing single element oxide powder with graphite powder, weighing and pressing the mixture. For a batch preparation of five pellets, the process takes about 15 to 20 minutes. The pellet is then put to the notch of the "sample holder" with a pair of tweezers. It goes through a drying cycle at 100° C for 2 seconds and charring cycle at 200° C for 1 second before it goes on to a vaporization cycle. The drying and charring cycle are necessary to eliminate the moisture and the gas in the pellet. At the vaporization step (1250° C, 5.0 seconds), the pellet is heated to incandescence and the analyte is vaporized. The vapor is carried to the plasma by an argon carrier gas. The transient atomic emission signals are usually narrow, well-defined peaks for the volatile elements studied.

A compromise carrier gas flow rate must be used for multielement analysis. The precisions (RSD 3-8% peak heights) are good for solid sample analysis. Linear calibration curves with a range of two to three orders of magnitude were established for Cd, Pb and Zn. The improvement factors in detection limits are 6 to 100 and 30 to 50 respectively in comparison to pellet-DSI and routine liquid sample nebulization.

For reference botanical samples, grinding (10 to 15 minutes in a mortar) is needed if the sample particles are too big. The preparation steps of pellets from these samples are similar to those for single element oxides except that the sample to graphite ratio is kept at 1 to 9 (w/w). Larger ratios result in poor signals or breaking of the pellet. Higher temperatures and longer periods are needed in the drying and charring steps to get rid of the organic matrix of the sample (Drying: 300° C, 60 sec; Charring: 400° C, 120 sec). The analyte concentrations in these

samples are read directly from the calibration curves established with single element oxides. For the two elements (Pb and Zn) monitored, it is found that some of the results have good accuracy, while others do not. The most interesting feature is that the precision in the vegetation samples is quite good, intimating that matrix matched standards or standard additions might provide good accuracy.

Table I: Analysis of MOE botanical samples

Sample type	(concentration in ppm)			
	Pb		Zn	
	Found	MOE	Found	MOE
V85-1	10+/-1	19+/-2.0	370+/-12	140+/-11
White birch	3.2+/-0.2	4	370+/-5	200
Norway maple	39+/-1	95	39+/-2	39

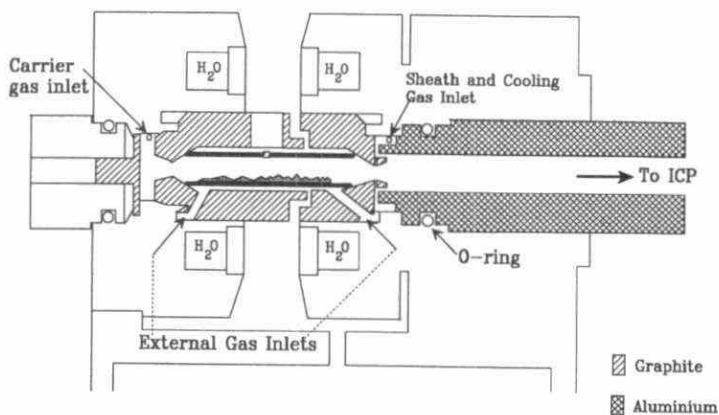
In conclusion, this system provides good precisions, improved detection limits and easy calibration using single element oxides. The procedure is simple and fast (10 minutes grinding, 15 minutes pellet preparation). The system is suitable for quick screening of large numbers of vegetation samples for volatile elements. Better results may be possible using more demanding methodologies such as standard additions or internal standards.

LESSONS LEARNED AND BEYOND.

The pellet DSI work has demonstrated that it is possible to analyze even very difficult samples with the DSI technique. The indications are that the same is true for pellet ETV. Unfortunately, when analyzing more difficult samples, the methodologies involved, standard additions and internal standards, may have to be accompanied with a chemical treatment as was done in our latter DSI work. This is unfortunate because it is very inconvenient to use these methodologies with solid materials. They are time consuming, difficult to automate and may introduce additional errors through contamination, particularly with the addition of matrix modifying chemicals. We believe that we must find convenient ways to break down the sample while it is in the solid form. The method should preferably not require solid handling and, ideally, will eliminate the need for the standard additions or internal standards methodology. With this in mind we have built an ETV solid sample introduction system which is specifically tailored to allow gases to be

used as reactants to break down the sample. The system is illustrated in Figure 3.

FIGURE 3



The system is most unique in one aspect, it provides a sheathing and "cooling" gas to mix with the gaseous effluent from the furnace. The sheathing gas will keep the analyte from condensing on the wall of the "after burner" tubing while the cooling gas is designed to cause turbulent mixing inside the after burner. Mixing should produce aggregates which will survive the transport to the plasma.

The chemistries possible are quite interesting. For example, gaseous halocarbons (Freon, carbon tetrachloride) can be introduced. On heating, highly reactive radical halides will be formed. These radicals could convert the sample to volatile halides which are vaporized much more easily. Even more interesting, it may be possible to operate the system in a "stop flow" mode. This should concentrate the analyte and allow sufficient time for slower chemical reactions to take place. The after burner also allows some opportunity for chemistry. The introduction of oxygen, for example, would encourage the rapid

formation of oxide aggregates, which are quite stable and have higher transport efficiencies than the corresponding halides.

CONCLUSION

We have learned that we are on the right track. The solution to the problem seems to be a more chemical approach, albeit gas phase, rather than a straight physical approach.

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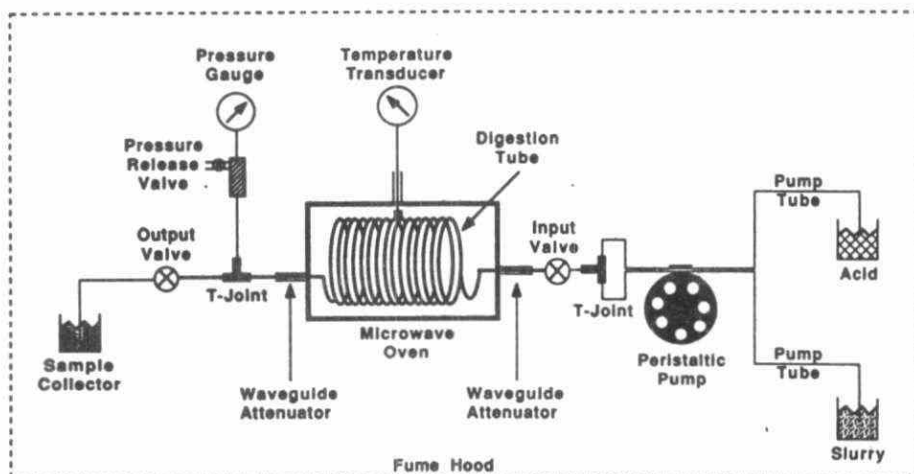
AN INTERRUPTED-FLOW MICROWAVE DIGESTION SYSTEM FOR ENVIRONMENTAL SAMPLE PREPARATION.

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In our laboratory we have worked for the last several years developing a microwave digestion system specifically designed to enhance throughput in the laboratory. The system is dramatically different from conventional microwave systems in that it uses a tube rather than a bomb or open vessel as the sample container. The configuration is illustrated in Figure 1.

FIGURE 1.



Sample is prepared in slurry form and then pumped through the tube into the microwave cavity. A conventional "kitchen" microwave oven is used. The tubing becomes a closed vessel when the valves are closed. Microwave energy is then applied for a period as short as two minutes. At the end of the two minute period, the tubing is allowed to cool for one minute and then the pressure is brought to atmospheric level by slowly opening the exhaust valve. The solution is then pumped into a container (or directly to an instrument) while a wash solution follows. The wash solution is then treated for approximately the same length of time. Results obtained from two NBS materials are presented in Table I.

Analysis of NBS Orchard Leaves and Bovine Liver

Percent Recovery

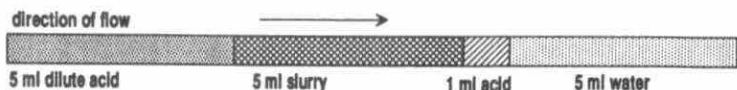
Element	Orchard Leaves	Bovine Liver
Al	29	35
Ba	89	50
Cu	92	100
Fe	80	107
Mg	100	111
Pb		97
Zn	100	98

They are generally quite good, with the exception of Al and Ba, which we discussed at length previously (1990 Technology Transfer Conference). They can be determined either by using extended digestion times or correction factors.

There are several ideas for improvement which resulted from our experiments with this first design. The first was the use of what one could call a segmented plug approach to the distribution of solutions in the tube. We have always been concerned about the escape of materials on the fringes of the acid plug. These would result in analytical errors

and also leave solid material which could result in memory effects as well as mechanical degradation of the valves. The solution has been to precede and follow the analyte/slurry plug with a water and then an acid plug. This means that any particles must escape through the acid plug, an unlikely occurrence since this will be a high concentration zone. The configuration is illustrated in Figure 2. An additional improvement has been implemented, a drop in volume from 50 to 25 ml. The system has been tested with NBS Orchard Leaves and results are similar to those which we obtained previously (Table I).

FIGURE 2

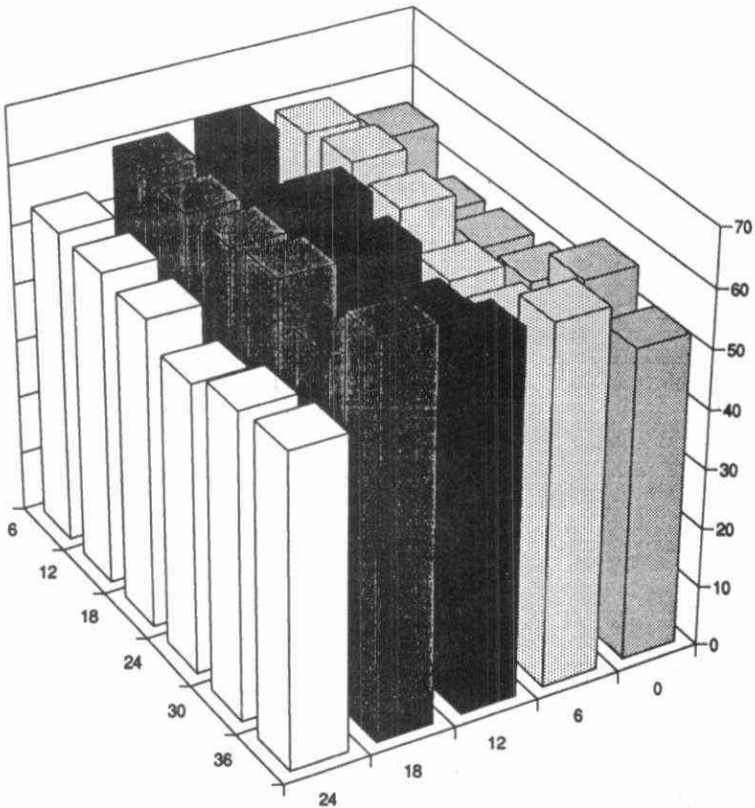


We have apparently eliminated memory effects by using an acid wash and rinse cycle. While this solves the analytical problems, it does bring up a question of throughput advantage. Considerable throughput is lost if the system is digesting only 50% of the time. To overcome this problem, we have been investigating the possibility of a multitube approach. In theory, a two tube system would have a throughput of approximately one sample every two minutes, while a four tube system would have a throughput of one sample every 1 minute. In the last case, the throughput would closely match the throughput of ICP spectrometer systems, however one must not forget that cooling and deaeration would require additional processing along the way, although they need not change the throughput since they could be done in-line.

It is critical that one appreciate that two types of "kitchen" microwave ovens are available at this time. Those with carrouseles and those with "spinners" at the top. Carrousel systems deliver power to only a small portion of the oven. The systems work because the material is brought through flux for uniform exposures by the rotating carrousel. This type of arrangement is unsuitable for our type of work, since only a small portion of the coil would be exposed. For this reason, spinner based systems must be used. One then must be concerned about the uniformity of flux inside the oven. To study what might happen in a spinner microwave oven, a series of power distribution experiments were conducted by placing beakers of a fixed volume of water inside a microwave oven. The power was turned on for a fixed length of time, and the change in temperature was used to obtain a measure of the power delivered to an aqueous sample. The data for a single beaker is presented in Figure 3. The vertical axis refers to relative power transferred as indicated by a change in temperature. The other two axes are physical dimensions from one corner. In essence, the "x and y" correspond to the floor of the oven.

Figure 1 indicates a roughly uniform distribution, however, it is interesting to consider what might happen when two coils were placed in the microwave, with one coil on or beside the other. One might then be concerned about shielding effects.

FIGURE 3



Figures 4 and 5 demonstrate various shielding experiments in which four beakers were placed in the corners of the microwave oven while another beaker was moved to various positions. It becomes clear that some shielding may occur. This may imply that a braiding arrangement is best, or simply that higher total power is required with more careful monitoring of the pressure within each tube.

FIGURE 4

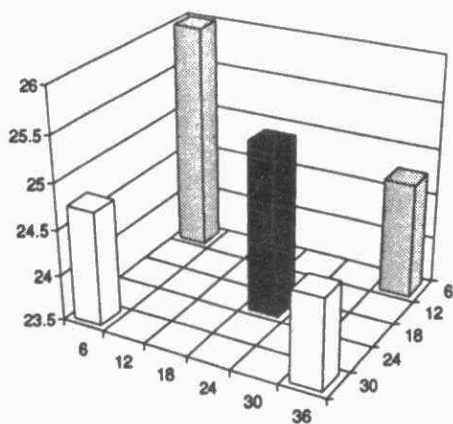


FIGURE 5

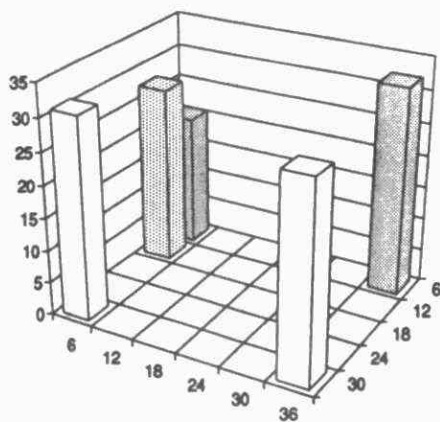


Figure 1 has been simplified somewhat. The present system actually incorporates feedback between the pressure transducer and the power input, thereby minimizing the potential for overpressures and damage to the equipment. When the pressure threshold is exceeded, power is cut to the system until the pressure is reduced to the threshold level. Only extremely rapidly changing pressure levels could now cause damage to the system. This would most probably be caused by using the wrong chemistry on a given sample type, an operator error.

Work continues towards the development of a fully automated system. We will discuss our work towards a "particle proof" valve at the conference.

DEVELOPMENT OF NEW CAPILLARY GC COLUMNS FOR DETERMINATION OF ORGANICS IN ENVIRONMENTAL SAMPLES. Thomas O. Tiernan*, John H. Garrett and Joseph G. Solch, Wright State University, Department of Chemistry, Dayton, Ohio 45435, U.S.A.

A multidimensional computer model has been developed which predicts the functional group composition of stationary phase coatings used in bonded-phase capillary GC columns which will yield optimum separations of selected mixtures of organic compounds. Among the variable parameters incorporated in this model are the temperature program, the head pressure, the column length, the column film thickness, and functional group interactions within the bonded phase. The model ultimately yields functional group capacity factors from which retention time data for separations of selected compounds are calculated for various stationary phase compositions. On the basis of these predictions, the optimum stationary phase is identified and synthesized, and a capillary column is coated with this phase and tested experimentally to evaluate its separation characteristics. The application of the model for predicting the separations of the entire set of the 2,3,7,8-chlorine substituted polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) isomers will be described. In addition, use of the model to predict separations of a set of volatile organic compounds on a series of commercially available columns of different lengths will be demonstrated. The performance of capillary columns prepared by J & W Scientific, our collaborator in this work, on the basis of the model predictions for both of these applications, will be discussed.

FLOW INJECTION PRECONCENTRATION COMBINED WITH DIRECT SAMPLE INSERTION FOR INDUCTIVELY COUPLED PLASMA SPECTROMETRY

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INTRODUCTION

The Inductively Coupled Plasma (ICP) has established an enviable performance record as a source for both atomic emission spectrometry (AES) and mass spectrometry (MS). While matrix effects in ICP-AES are only moderate, it lacks the sub part-per-billion (ppb) detection limits of ICP/MS. On the other hand, ICP/MS suffers from a wide variety of matrix effects [1] which can affect the accuracy of the system. In our laboratory we have experimented with Direct Sample Insertion (DSI) [2] for liquid sample analysis by ICP-AES and ICP/MS. While detection limit advantages are gained with DSI in both AES and MS, DSI-ICP/MS offers the additional advantage of a significant reduction in matrix effects by eliminating the solvent from the sample introduction process [2].

Flow Injection (FI) has been used extensively in configurations commonly called Flow Injection Analysis (FIA) systems [3], and more recently as an approach to extending the capabilities of atomic spectrometry [4]. FI preconcentration offers two important benefits for ICP spectrometry. The first is the preconcentration advantage, generally between one and two orders of magnitude [5-8]. The second advantage arises from the separation of the analytes from interfering matrix constituents. Samples which have undergone the FI preconcentration

treatment emerge from the system in the same matrix, namely, that of the eluant. This has advantages for ICP spectrometry, particularly ICP/MS, which is more sensitive to matrix effects than ICP-AES.

One loses a great deal of the advantage of preconcentrating the sample if a significant fraction does not reach the plasma. The low transport efficiency (between 1-5%) of most nebulizer/spray chamber combinations indicates that a special advantage for FI-ICP spectrometry may be gained by using DSI, which provides a sample introduction efficiency of 100%. While previous liquid DSI-ICP-AES and DSI-ICP/MS work in our laboratory was oriented towards the use of wire loops [2,9], there have been several recent reports in the literature that either a thin-walled thin-stemmed graphite cup or a deep undercut electrode give excellent performance with liquid samples [10,11]. These configurations reduce cup mass and facilitate rapid heat transfer to the sample, resulting in narrower, more intense peaks. In our previous report [12] we discussed both the development of special graphite probes for FI-DSI-ICP-AES and the experimental aspects of the method.

In this paper we have addressed the question of the expected "multiplicative effect". It would seem that the preconcentration factor of the FI front end could be almost directly multiplied by the detection limit improvement of DSI-ICP. Since both advantages are significant, about a factor of 10, the combination of the two should provide a stunning improvement, of about two orders of magnitude.

RESULTS AND DISCUSSION

The operating parameters for the flow injection system and the DSI system were optimized separately, as there was no reason to expect any interaction between the two subsystems. The Chelex-100 ion exchange resin columns were optimized with respect to length and inner diameter. "Optimal" in this case was determined to be the column that gave the highest intensity signal in the smallest time window for a fixed sample volume without exceeding the loading capacity of the column. The other operating parameters that were varied include sample loading rate, pH of the buffer, acid strength and elution flow rate. These experiments were normally conducted by running the eluant directly into a nebulizer/spray chamber system.

The precision of determinations at the 5 ppb level (about 100 times the detection limit with clean blanks) using peak integration for the three elements averaged 4% RSD using manual drying of the eluant into a cup. Although this precision is quite good, there is potential for improvement since the DSI system has previously exhibited precisions for liquid work of about 1% [9] when automated.

By collecting small fractions of eluant, it was determined that a 5.0 mL injection volume eluted in approximately 160 μ L providing a preconcentration factor of 30, typical of many of the systems described above. Detection limit improvements ranging from $\times 10$ to $\times 100$ (element specific) have been reported [8] for DSI alone. In Table 1 we have presented detection limits for DSI obtained independently of the FI system. One would then expect that the product of the DSI Improvement Factor and FI Preconcentration Factor would produce detection limit improvements of the magnitudes shown in Table 1. The performance data obtained are given in Table 2. The thin walled cup detection limits of Table 1 were obtained with a slightly different optical arrangement (viewing height and lens position) than that used for the final set of

experiments which provided the data in Table 2. Nevertheless, the observed multiplicative effect is certainly of the same order as that predicted.

Table 1. FI-DSI-ICP-AES Performance Expectations

Element	Nebulizer Detection Limit (ppb)	DSI Thin Cup Detection Limit (ppb)	DSI Improvement Factor	FI Preconcn. Factor	Expected Improvement Factor
Pb	30	0.9	30	30	900
Zn	80	0.6	130	30	3900
Cu	8	0.9	9	30	270

Table 2. FI-DSI-ICP-AES Performance Data

Element	Nebulizer Detection Limit (ppb)	Ideal Blank Detection Limit (ppb)	Improvement Factor	Real Blank Detection Limit (ppb)	Improvement Factor
Pb	30	0.05	600	0.04	700
Zn	80	0.07	1200	1	80
Cu	8	0.06	140	0.2	40

As the relatively high nebulizer detection limits in Table 2 reveal, the spectrometer could be considered somewhat sub optimal. Several nebulizers (Meinhard and Légère) imported from a conventional spectrometer provided the solution detection limits presented when used with this system, yet these nebulizers performed up to factory specifications on a newer system, usually providing detection limits about an order magnitude better. Since the purpose of the experiments was to establish the general validity of the multiplicative effect, we are extremely pleased with our results. It is however unfortunate that the optics had not been brought up to optimal at the time of these experiments. None-the-less, detection limits in the 50 ppt range are encouraging at this stage.

Since we do not have an in-house ICP/MS system, this was our first experience working at sub ppb levels. Our first experiments with the system demonstrated that the blank levels were very high. After several changes of reagents and the installation of guard columns, the blanks were reduced drastically, but continued to be quite high, with signals corresponding to 0.2, 3 and 0.8 ppb of Pb, Zn and Cu respectively. Some detective work revealed that both the valves on our injection system, which has been in use for two years, were contaminating the system. One column of the data in Table 2, labelled "real", was collected using the less contaminated of the valves. The first set of detection limits, called "ideal", was determined using doubly distilled water in a DSI cup as the ideal blank. The improvement factors were calculated using both sets of data.

We have made no attempt in this study to demonstrate the linearity of calibration curves. Both separations methodologies [13] and DSI technologies [9] have demonstrated a linear response over at least three orders of magnitude. While this would indicate that curves would be linear, it will be critical to evaluate the performance when we move on to more extensive testing. We have also not attempted to further reduce detection limits by increasing the sample volume. Elementary theory indicates that a linear improvement would be expected as long as the column loading capacity was not approached. If implemented under the same operating conditions, the price paid would be a proportional increase in analysis time.

CONCLUSIONS

Considerable effort on our part was expended during the earlier stages of this work to develop a convenient automated FI-DSI interface. Our efforts were primarily directed at depositing the FI eluant on wire loops, which we felt would provide the best detection limits. The difficulty of achieving this interface led us to change to graphite electrodes as probes. The excellent wire loop detection limits were, in large part, due to the rapid heating of the loop. This observation encouraged us to develop an extremely thin walled, narrow stem electrode. This does not imply that graphite cups are necessarily the ultimate solution. Our expectation is that a system which sprays the eluant onto a hot surface would provide a suitable interface. The surface could be either a DSI probe or an electrothermal vaporization (ETV) system. The ETV configuration offers a number of potential advantages which are worth mentioning.

1. Convenient control of the drying step since the capability is integral in the design.
2. Rapid switching between conventional liquid introduction and ETV introduction.
3. More control of the experiment by decoupling the vaporization and excitation systems.

It is important to note that some elements generally do not provide the excellent performance of the elements selected for this test, due to the formation of non-volatile carbides. This is not to say that these elements can not be determined by this approach; there appear to be at least two ways to address the problem. The first is to use metallic DSI probes [9,11] or ETV boats. There would then be some concern that the acidic solutions used with an ion exchange preconcentration column would corrode the probe and introduce significant contamination. There are several FI preconcentration modes [8,14] which elute the analyte in a

complexed form after either an ion exchange or an adsorption column. The eluants are considerably less corrosive than the strong nitric acid that is used for traditional ion exchange. Alternatively, if the complex can survive the drying process on a graphite surface, it may provide a volatile carrier for the bound metals, thereby furnishing even better performance (sharper peaks) while reducing concerns about carbide formation.

We are pleased to observe the multiplicative effect that one would expect with a complementary hybrid arrangement of this type. FI-DSI-ICP spectrometry seems to offer the potential for bringing ICP/MS detection limits to ICP-AES, as well as providing the other advantages of FI sample handling. While not demonstrated in this work, a natural by-product of the separation phenomenon would be a reduction of ICP/MS matrix effects, perhaps to levels matching or less significant than those of ICP-AES. One would also expect detection limit benefits for FI-DSI-ICP/MS to accrue.

DSI systems have demonstrated relative standard deviations of less than 1%. Similar performance is possible with FIA systems. Our precision of 4% leads us to believe that there are significant advantages to be gained in detection limits, reproducibility, and general operating performance, by developing an automated interface between the FI and the DSI systems. In fact, an automated interface would seem to us to be one of the most important obstacles to overcome before this technique can achieve a wide level of acceptance.

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AUTOMATABLE TOTAL CYANIDE ANALYSIS.

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The analytical determination of the "total cyanide" concentration is very important for many industrial applications and for environmental protection, regulation and process control. Gold and silver extraction by cyanidation and froth flotation are the principal industrial applications for cyanide. According to Von Michaelis (1), about 41% of the cyanide consumed in gold and silver recovery is consumed in South Africa where about two thirds of the "free" world gold is produced, while Canada consumes about 7.1% of the world cyanide consumption. Since the simple cyanide and some of the metallocyano complexes are toxic or extremely toxic, they are subject to governmental regulations for worker safety and environmental discharge. According to the Ontario regulations, the maximum acceptable level of cyanide for drinking water is 0.2 mg/L (2). The discharge of cyanide in South Africa is regulated in terms of "total" cyanide with a maximum allowable level of 0.5 mg/L (3). The U.S. Environmental Protection Agency (EPA) water quality criterion to protect freshwater aquatic life is 3.5 $\mu\text{g/L}$ of free cyanide as a 24-hour average, and the concentration should not exceed 52 $\mu\text{g/L}$ at any time (4).

The term "total cyanide" is used for all cyanides, i.e. free as well as coordinated cyanide in a sample. The concentration of free cyanide in a solution depends on the pH value of the solution and on its content of heavy metals capable of forming cyanide complexes (5). Depending on their stability constants, complexed metal cyanides vary in toxicity. The ionization of such complexes, changes in pH, or photo-decomposition caused by sunlight, can result in the formation of molecular hydrogen cyanide which is a very toxic gas.

Recently, "total cyanide" determination has become the subject of numerous published papers, which include the use of a variety of methods such as titrimetry, colorimetry, cyanide-selective electrodes, coulometry, gas chromatography, high performance liquid chromatography, polarography, and atomic absorption, but still an appropriate method free from matrix interferences is not available. Liu et al (6) emphasize that the standard methods for cyanide analysis use acid distillation procedures to decompose metal cyanide complexes, but these methods have several drawbacks. The acid distillation procedure is very time-consuming and labour intensive. The methods also suffer seriously from a number of interferences such as sulphide and thiocyanate. To avoid the problems and safety handling aspects, automatable methods would be preferred. Some automated methods have been suggested by many authors, but most still are preceded by a tedious manual distillation procedure if the "total cyanide" value is desired. Zhu and Fang (7) stress that stringent control of cyanide in industrial effluents demands automation so that determinations can be repeatedly conducted preferably at intervals of a few minutes for extended periods. These authors claim that the serious potential hazard of cyanide exposure can be lowered if the distillation process is not incorporated in the system.

Liu et al (6) have suggested a different approach to cyanide detection based on the photodissociation reaction of metal cyanide complexes under UV irradiation in an acidic medium using an automated system. Many authors have shown that metal-cyano complexes can undergo photodisruption when they are exposed to ultraviolet irradiation, but stable cyano-complexes such

as $\text{Fe}(\text{CN})_6^{4-}$, $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Co}(\text{CN})_6^{3-}$ are difficult to completely photodissociate. This cyanoligand displacement from the coordinated cyanide does occur with UV irradiation, but UV irradiation can also result in the decomposition of thiocyanate to form sulphide and cyanide or can affect other sulphur species present in the solution. Special precautions must be taken to prevent these interferences which could produce a positive interference. Sample pretreatment is an important procedure to prevent interferences. Nonomura (8) has reported an optimized analytical procedure for the determination of total cyanide which avoids this positive thiocyanate interference.

The ultraviolet photochemical decomposition combined with the Ion Chromatography (IC) method, has been used as the basis for our research. Electrochemical detection (EC) is an appropriate way of detecting low amounts of simple/free cyanides in solution. One of the advantages of using IC with EC detection is the low amount of sample required and the rapid response obtained, as well as the sensitivity of detection. UV spectrophotometric detection was used with the IC in order to check or confirm the metal-cyano complex disappearance. In addition, a cyanide specific ion electrode was used for simple cyanide detection. In order to achieve an optimal photodecomposition, the research is focused on the analysis of the specific wavelength photosensitivity of the cyanide complexes and optimized reducing conditions.

EXPERIMENTAL SECTION

REAGENTS. Standard Cyanide Solution. 1000 mg CN/L of NaCN in 0.01M NaOH, prepared from analytical grade reagents and deionized water. Working solutions were prepared by dilution. Standardization was carried out using AgNO_3 .

Standard Ferrocyanide Solution. 100 mg CN/L of $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ (99% purity) in 0.01M NaOH for IC method and 0.1M NaOH for the specific ion electrode method for ionic strength adjustment, prepared from analytical grade reagents and deionized water. Working solutions were prepared by dilution.

Standard Ferricyanide Solution. 100 mg CN/L of $\text{K}_3[\text{Fe}(\text{CN})_6]$ (99.92% purity) in 0.1M NaOH for the specific ion electrode method for ionic strength adjustment, prepared from analytical grade reagents and deionized water. Working solutions were prepared by dilution.

ELUENTS.

Eluent 1. 0.01M NaOH prepared from analytical grade reagent and deionized water.

Eluent 2. 20 mM NaOH, 15 mM NaCN and 30 mM NaClO_4 (9).

EQUIPMENT. A Dionex 2000 Ion Chromatography was used, having an AS5 separating column and an electrochemical cell or a UV spectrophotometer after the column. A loop sample size of 10 μL was used in the injection valve and an eluent flow of 2 ml/min was set.

A Dionex potentiostat was set-up with an applied potential 0.15 V vs. Ag/AgCl and the output was sent to a recorder for peak height measurement.

A Waters Millipore spectrophotometer, model 481, was used at 0.05 AUFS with a specific wavelength for each metal cyano complex.

An Orion cyanide electrode, model 94-06 was used with an Orion specific ion meter, model 720A.

An Oriel 500 Watt mercury lamp, model 6285, was operated at 74 volts and 5.3 amperes as the UV irradiation source.

GENERAL PROCEDURE. 10 ml of fresh solutions of ferri or ferrocyanide prepared by dilution from the stock solution, having either 7 mg CN/L for IC measurements or 10 mg CN/L for the specific ion electrode measurements, were irradiated with UV for a period ranging from 1 to 15 minutes. The free cyanide produced by photolysis was detected in the solution using both methods (specific ion electrode detection and IC using both EC and UV detection). With the IC method using EC detection, eluent 1 was used. The remaining metal-cyano complex, was checked using IC with UV detection, and eluent 2 was used. For the specific ion electrode measurements ionic strength adjustment was achieved by diluting the ferri or ferrocyanide stock solution with 0.1 M NaOH to 10 mg CN/L.

For the effect of temperature on ferrocyanide solutions, 10 ml of fresh solutions of ferrocyanide prepared from the stock solution, having 7 mg CN/L, were held in a bath at 5°C or 45°C and were UV irradiated.

RESULTS AND DISCUSSIONS.

CALIBRATION CURVES. The standard calibration curve for cyanide measurement using the specific ion electrode shown in Figure 1(a), illustrates linearity between millivolts and concentration of the cyanide solution from 0.5 to 20 mg CN/L. Data follows the regression curve: $\log(\text{conc.}) = 0.016818 * \text{mV} - 2.003036$, where concentration is given in mg CN/L. The correlation coefficient is 0.9978 and concentrations were determined with in $\pm 3.7\%$. A typical calibration curve for cyanide measurement using IC with EC detection is shown in Figure 1(b), having a correlation coefficient of 0.999 and concentrations were determined with in $\pm 0.5\%$. For the measurement of the unknown solutions of photodissociated cyanide using IC with the EC detection, a calibration curve was prepared immediately before the measurements because of day to day variability in the calibration curve. No linear region was found for these calibrations.

The standard calibration curve for ferrocyanide measurements using UV spectrophotometric detection is shown in Figure 2(a), and it shows linearity between peak size (mm) and cyanide concentration (mg/L). Data follows the regression curve: $\text{CN}^- \text{ conc. (mg/L)} = (\text{peak} - 0.411966) / 7.244083$, where peak size is given in millimetres. The correlation coefficient is 0.9983 and concentrations were determined with in $\pm 4\%$.

UV IRRADIATION OF SODIUM CYANIDE. When the sodium cyanide solution was U.V. irradiated, a constant value of cyanide was obtained using both methods for the detection of cyanide as shown in Figure 2(b). The CN⁻ concentration of the solution for specific ion electrode was 10 mg/L and for the IC with EC detection 7 mg/L. This shows UV irradiation that the

simple cyanide was not photosensitive to UV irradiation under the test conditions and that both detection methods were suitable for free cyanide determination.

UV IRRADIATION OF FERROCYANIDE SOLUTION. Solutions of potassium ferrocyanide with pH about 12 were UV irradiated for various times and the amounts of free cyanide produced were measured by both IC with EC and the specific ion electrode. The results are presented in Figure 3 as percentage free cyanide recovered. In our experiments, 98% of CN^- recovery was measured after 5 minutes of UV irradiation, with the use of the specific ion electrode method; and 100% recovery with the use of the IC method with the EC detection. The disappearance of ferrocyanide was followed with the IC using UV spectrophotometric detection set at 220 nm, and complete disappearance of ferrocyanide was found after 10 minutes of radiation (see Figure 3). A gradual photodissociation of the metal complex was observed with time. According to many authors, ferrocyanide changes rapidly to the $(Fe(CN)_5H_2O)^{3-}$ with the liberation of the cyanide ligand, however in our work complete photodissociation was found after 10 minutes showing a complete disappearance of $(Fe(CN)_5H_2O)^{3-}$. Otake et al.(10), claim that the rate of disappearance of ferrocyanide and $(Fe(CN)_5H_2O)^{3-}$ were zero order with respect to the concentration of the species and both rates were independent of reaction temperature, but varied with the pH value and the light quanta absorbed by the solution. The use of the UV spectrophotometer confirmed the fact that ferrocyanide dissociated. This ferrocyanide dissociation can be accelerated if the UV intensity is increased by increasing the applied voltage to the Hg lamp.

EFFECT OF TEMPERATURE ON UV IRRADIATION OF FERROCYANIDE. The time of radiation causes a temperature rise in the ferrocyanide solution. This gradual increase in temperature with time is shown in Figure 4. This variation in the temperature is important because the rate of ferrocyanide photodissociation may be temperature dependent. For this reason, the rate of ferrocyanide photodissociation was studied at different temperatures. Contrary to Otake et Al's work, the rate of photodissociation for the ferrocyanide solutions increased with the temperature of the solution as shown in Figure 5. For the solution held at a temperature of 5°C, 90% of ferrocyanide dissociation was achieved at 10 minutes. For the solution held at 45°C, that photodissociation was achieved at 4 minutes (see Figure 5). Thus, elevated temperatures could be used to reduce irradiation time.

U.V. IRRADIATION OF FERRICYANIDE SOLUTION. A solution of potassium ferricyanide with a pH about 12 was UV irradiated and the free cyanide produced was detected with the specific ion electrode method. A maximum of about 80% of cyanide recovery was obtained after 7 minutes and decreased to 72% after 15 minutes. Since the recovery of ferricyanide was only 80% but the recovery of ferrocyanide was quantitative, the use of a reducing agent was indicated. Various reducing agent were tested (sodium borohydride, 0 to 250 mg/L of ferricyanide solution; 50% v/v hypophosphorous acid, 0% to 10% v/v in the ferricyanide solution; and hydrazine, 0.1 to 100 mg/L of ferricyanide solution). The highest recovery was found with 0.5% of 50% v/v hypophosphorous acid. The results with this reducing agent are shown in Figure 6. After 10 minutes of UV irradiation, 98% cyanide recovery was achieved.

CONCLUSIONS.

The following conclusions are based on the initial results of this study.

- (1) The specific ion electrode had a linear response over cyanide concentrations from 0.5 to 20 mg/L. Concentrations were determined with in $\pm 3.7\%$.
- (2) IC with EC detection did not show a linear response even over a relative narrow CN^- concentration range 0 to 7 mg/L. A smaller size of the micro-loop may be needed to obtain linearity. A calibration curve must be performed with the measurements since the calibration varies from day to day. Concentrations were determined with in $\pm 0.5\%$ in most of the calibrations.
- (3) IC with UV spectrophotometric detection had a linear ferrocyanide response over the range 0 to 7 mg CN^-/L . Concentrations were determined with in $\pm 4.0\%$.
- (4) No UV photodecomposition of simple CN^- was observed when sodium cyanide solutions were UV irradiated.
- (5) Quantitative yield of cyanide recovery was found for ferrocyanide solutions after 10 minutes of UV irradiation.
- (6) Temperature could be used to increase rate of cyanide recovery from ferrocyanide solutions.
- (7) Quantitative yield (98%) of cyanide recovery from ferricyanide solutions required a reducing agent. The best reducing agent tested was 0.5% of 50% v/v hypophosphorous acid.

FUTURE WORK.

This project will a) demonstrate that quantitative decomposition of other metal cyanide complexes can be accomplished without causing cyanide loss, b) that the thiocyanate ion is not decomposed under the required radiation conditions, c) that there are no other interferences that bias the total cyanide values, and d) describe an appropriate automatable system.

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FIGURE 1(a): CYANIDE CALIBRATION
USING SPECIFIC ION ELECTRODE

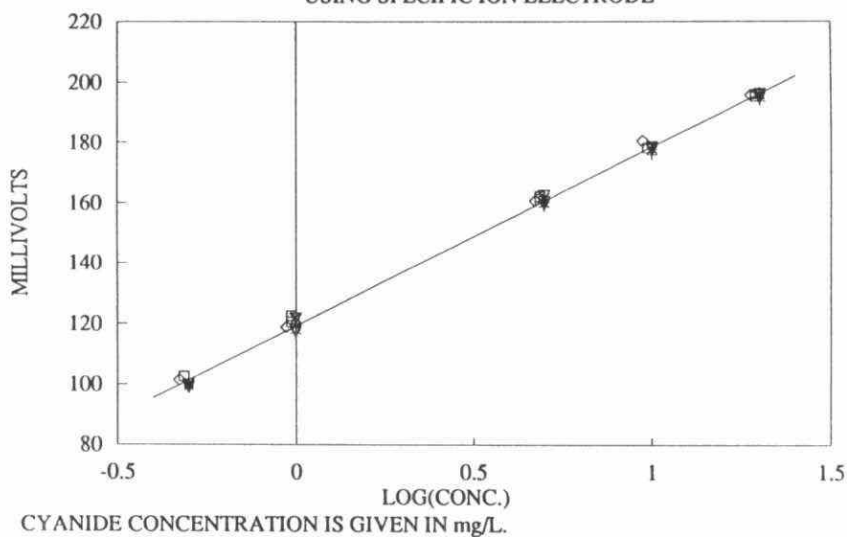


FIGURE 1(b): CYANIDE CALIBRATION
USING ELECTROCHEMICAL CELL

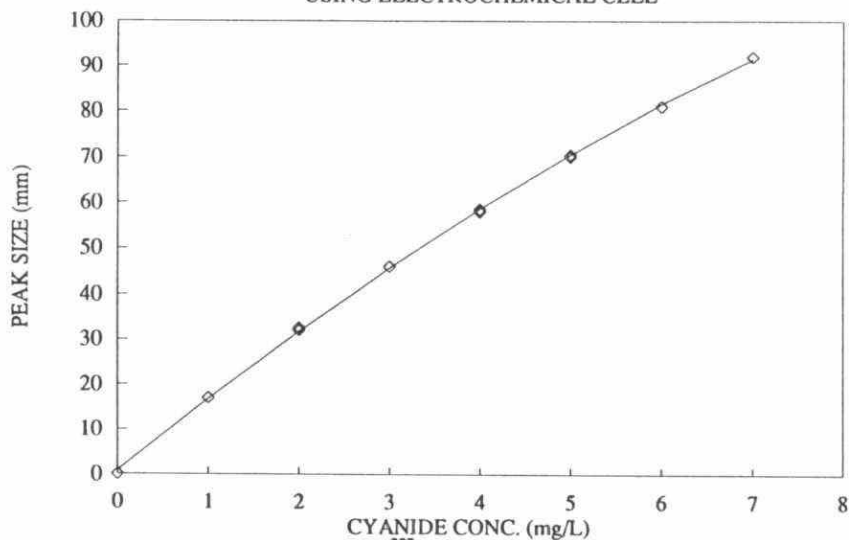


FIGURE 2(a): FERROCYANIDE CALIBRATION
USING UV SPECTROPHOTOMETRIC DETECTOR

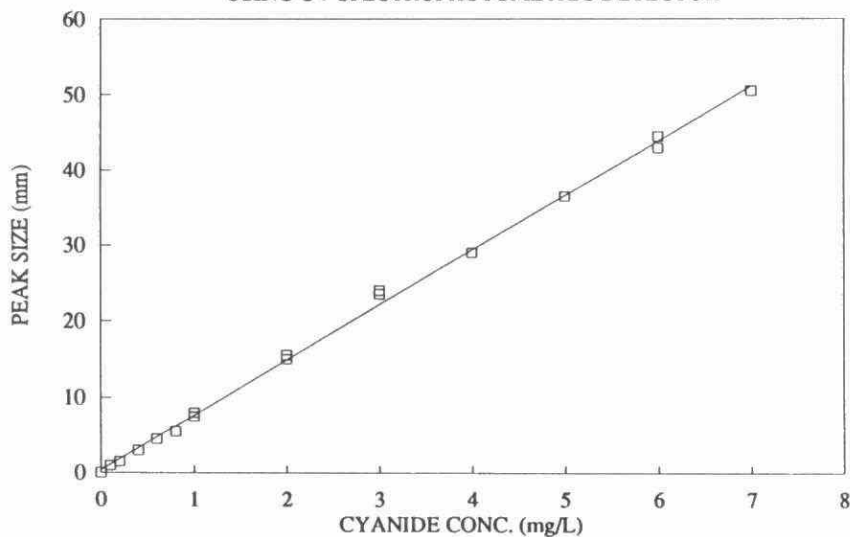


FIGURE 2(b): CYANIDE UV IRRADIATION
USING SPEC. ION ELECTRODE AND EC CELL

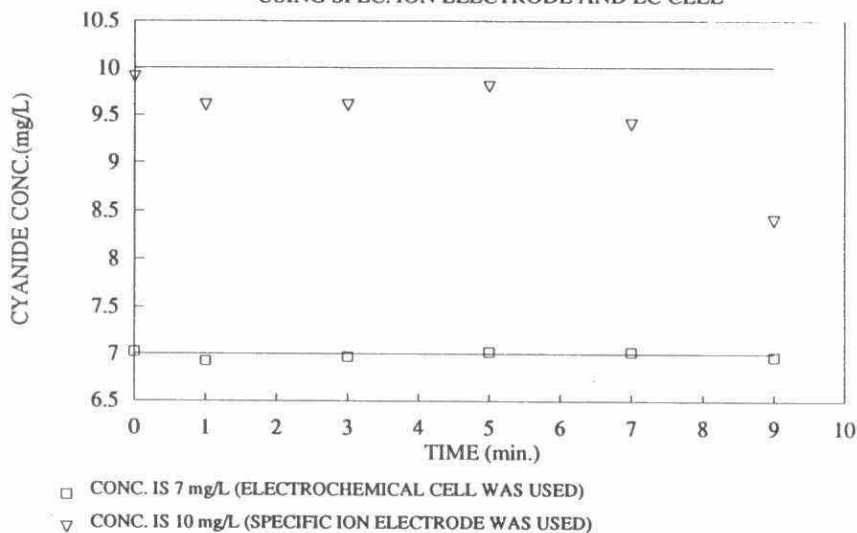


FIGURE 3: FERROCYANIDE UV IRRADIATION
DISSOC. AND NON-DISSOCIATED CYANIDE AFTER UV IRRADIATION

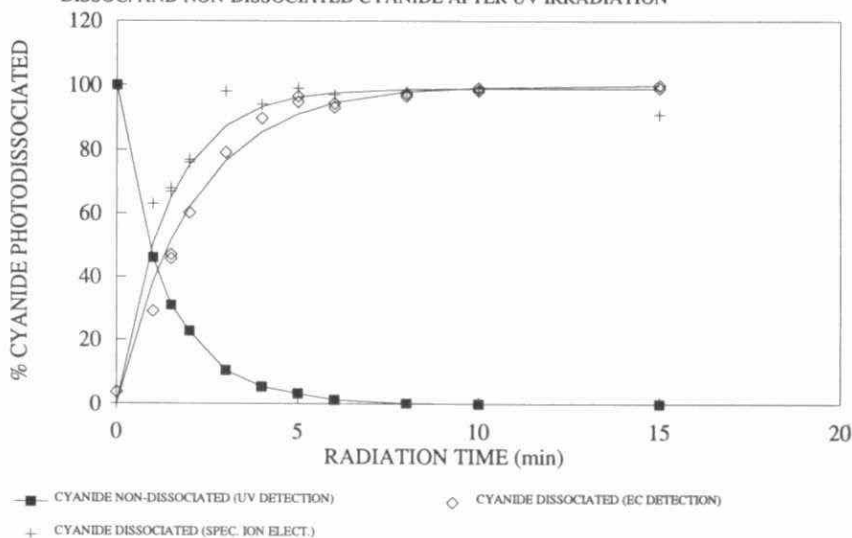


FIGURE 4: TEMPERATURE-IRRADIATION TIME
FERROCYANIDE SOLUTION

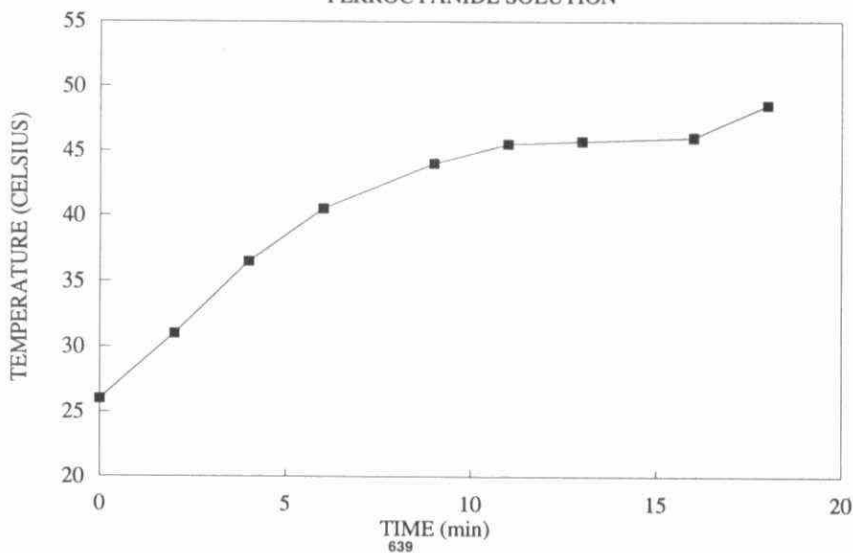


FIGURE 5: FERROCYANIDE PHOTODISSOCIATION
CYANIDE DETECTED USING ELECTROCHEMICAL CELL

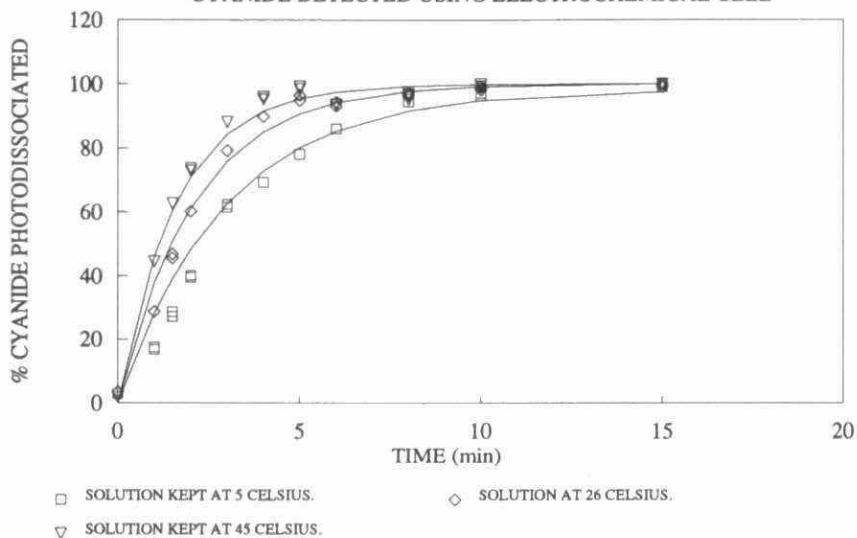
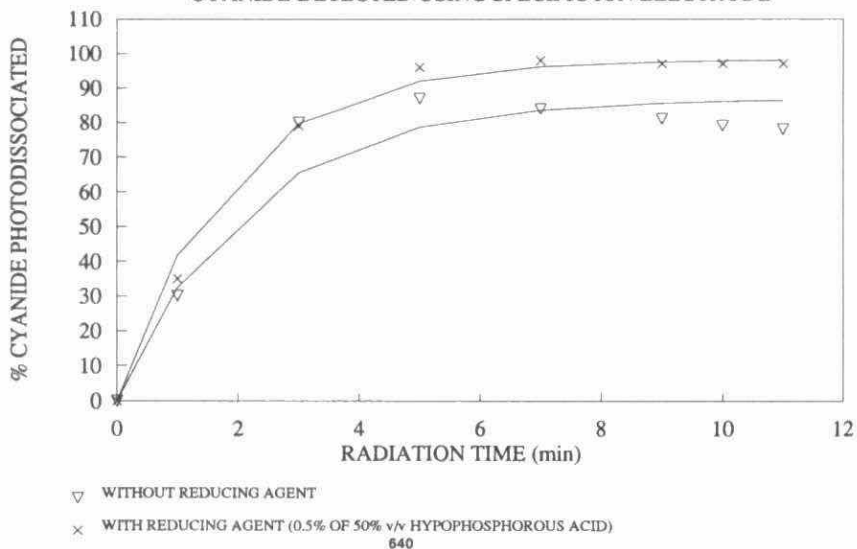


FIGURE 6: FERRICYANIDE PHOTODISSOCIATION
CYANIDE DETECTED USING SPECIFIC ION ELECTRODE



THE USE OF ENVIRONMENTAL ISOTOPE SURVEYS IN ASSESSING CONTAMINATION POTENTIAL OF "CONFINED" AQUIFERS

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ABSTRACT

The "freshwater aquifer" is a widely-used, regional aquifer which underlies most of Essex, Kent, and Lambton Counties in southwestern Ontario. It consists of fractured bedrock subcrop plus a thin layer of overlying granular material. Even though the aquifer in the central part of Essex County is remote from the obvious recharge area and is covered by more than 25 m of clayey overburden, it contains groundwater that appears to be less than about 40 years old (Cmokrak, 1991). This enclave of anomalously young groundwater coincides with a linear morphological feature mapped by Morris (pers. comm.). We used a program of exploratory drilling, hydrometric monitoring, environmental isotope tracing, and natural geochemical tracing to determine if the feature can explain the rapid recharge of the freshwater aquifer. This paper outlines the problem and briefly summarizes our initial results.

INTRODUCTION

Objectives

The main goal of this study is to determine whether a linear morphological feature, reported to be an esker buried beneath clayey overburden, is responsible for the anomalously young groundwater noted in the central part of the freshwater aquifer in Essex County, Ontario. The primary objectives in the first year of the study were to: (1) verify the existence of the buried esker reported by Morris (pers. comm.), (2) verify the existence of the anomalously young groundwater in the freshwater aquifer, and to (3) examine the spatial relationship between the young groundwater and the feature.

The Problem

The "freshwater aquifer" is a widely-used, regional aquifer which underlies most of Essex, Kent, and Lambton Counties in southwestern Ontario. Regionally, the aquifer consists of fractured bedrock subcrop and generally not more than a few metres of granular material. The aquifer thickens considerably in bedrock valleys such as the one in Sarnia (Intera Technologies Ltd., 1987). Dillon (1987) estimate that the horizontal groundwater velocity in the freshwater aquifer in central Essex County is in the order of 2.5 m/a.

The clayey overburden overlying the freshwater aquifer commonly exceeds 30 m. According to Desaulniers et al. (1981), this overburden is usually thick enough and of sufficiently low

permeability (in the order of 2×10^{-10} m/s) to be widely suitable for waste disposal. Ruland et al. (1991) conclude that active groundwater flow in the clayey overburden is restricted to the upper several metres. Recent research by D'Astous et al. (1989), however, raises some doubt about our ability to accurately characterize the hydraulic conductivity of the fractured clays that commonly occur throughout the region.

At the University of Windsor, we have mapped the distribution of environmental isotopes (oxygen-18 and tritium) in the freshwater aquifer in Essex, Kent, and Lambton Counties. The distributions of these isotopes reflect the natural recharge, discharge, and flow directions in the aquifer. The distinctive input functions of these isotopes can be used to differentiate between groundwater recharged thousands of years ago and groundwater recharged within the last few decades. Fritz and Fontes (1980) and others give details about the use of oxygen-18 (^{18}O) and tritium (^3H) in hydrogeologic studies.

Figure 1 from Crnokrak (1991) shows the distribution of ^{18}O in the freshwater aquifer in Essex County. ^{18}O analyses are expressed in the standard δ notation in parts per mil (o/oo) difference relative to the international standard SMOW (Standard Mean Ocean Water) (Craig, 1961), where: $\delta^{18}\text{O} = [(R_x - R_{\text{SMOW}})/R_{\text{SMOW}}] \times 1000$; where: $R = ^{18}\text{O}/^{16}\text{O}$ and $x = \text{sample}$. The analytical precision for ^{18}O measurements by mass spectrometry is better than ± 0.2 o/oo.

Figure 1 is based on groundwater collected from 35 domestic wells completed in the freshwater aquifer. According to Crnokrak (1991), Desaulniers et al. (1981), and Edwards and Fritz (1988), the $\delta^{18}\text{O}$ values in the northeast and northwest parts of the county (-15 and -16 o/oo) indicate groundwater that was recharged during cooler climatic conditions more than 10,000 years ago. The relatively enriched $\delta^{18}\text{O}$ values found in the groundwater in the southern and central parts of the county (-8 to -10 o/oo) are indicative of groundwater recharged under the milder climatic conditions of the past 10,000 years.

Crnokrak (1991) reports that the electrical conductivity (EC) and ^3H distributions are consistent with the ^{18}O distribution. She observed EC values in the freshwater aquifer greater than $3000 \mu\text{S}/\text{cm}$ in the northeast and northwest parts of the county, while the EC values are less than $500 \mu\text{S}/\text{cm}$ where the relatively enriched $\delta^{18}\text{O}$ values occur.

More significantly, the groundwater in the enclave of enriched $\delta^{18}\text{O}$ values in central Essex County have ^3H concentrations characteristic of recharge since 1952. Groundwaters recharged since 1952 contain anthropogenic ^3H derived from atmospheric testing of nuclear bombs. These groundwaters are characterized by ^3H concentrations exceeding 3 TU, where: 1 TU = 1 tritium unit = 1^3H in 10^{18} ^1H atoms. Groundwaters recharged prior to 1952 contain only cosmogenic ^3H that has now been reduced by radioactive decay to concentrations less than 3 TU.

The location of this enclave of young groundwater is puzzling. Rapid vertical recharge through the thick aquitard has been considered to be unlikely in view of the isotopic studies

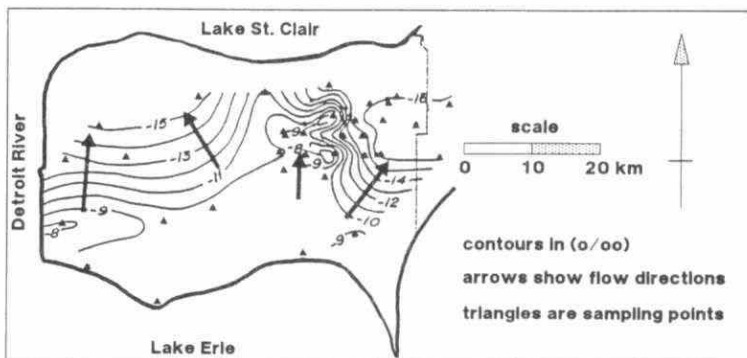


Figure 1. Distribution of oxygen-18 in the freshwater aquifer (after Crnokrak, 1991).

by Desaulniers et al. (1981). Lateral flow from the closest obvious recharge area also seems improbable given the low groundwater velocity in the aquifer reported by Dillon (1987).

Although Crnokrak's (1991) ^{18}O distribution map for the freshwater aquifer may be explained using the groundwater velocity reported by Dillon (1987), her ^3H distribution map cannot be explained in this manner. Groundwater containing anthropogenic ^3H should only be found within a few hundred of metres of the recharge area. The nearest surface expression of the aquifer recharge area, however, is more than 10 km upgradient from some of the tritiated groundwater in the freshwater aquifer reported by Crnokrak (1991).

A clue to the origin of this young water may lie in the study by Morris (pers. comm.). Based on remote sensing, field mapping, and drilling data, Morris (1989) mapped a linear morphological feature in central Essex County that he concludes is a buried esker. Morris (1989) first noted the feature by lighter tones on remote sensing images. There are no other obvious surface expressions of this feature. While comparing data in the summer of 1989, we noted that Morris' linear morphological feature coincided with Crnokrak's enclave of anomalously young groundwater in the freshwater aquifer.

We began with two hypotheses to explain the young groundwater. First, groundwater velocities are very high along the reported esker enabling rapid lateral groundwater movement from the closest obvious recharge area. Second, precipitation has recharged the freshwater aquifer rapidly by infiltrating through localized deep vertical fractures through the aquitard which are related to the settlement of the clayey deposits over the buried esker.

METHODS

Our general approach to the problem has been to assess groundwater ages and flow directions along, and perpendicular to, the linear morphological feature. To do this, we examined ^{18}O , ^3H , EC, and hydraulic head distributions in the vicinity of the feature. We installed monitoring wells in two transects across the feature (Sites 1 and 2 on Figure 2) and extended the network with nearby domestic wells. Figure 2 also plots the trace of the linear morphological feature reported by Morris (pers. comm.).

At both Sites 1 and 2, we attempted to drill a "deep" hole through the crest of the feature and on to bedrock to determine the stratigraphy and the depth to the top of the aquifer. Since the feature has no surface expression, this proved difficult. After installing a monitoring well in the deep hole, we completed the transect with a two or three level piezometer nest and one or two additional wells screened in the aquifer within a distance of about 200 m.

We squeezed porewater from Shelby tube samples of clayey overburden that were taken every 3 m from one deep hole at each site. These samples were used to determine the vertical distribution of ^{18}O . Samples from the monitoring and domestic wells were analyzed for ^{18}O , ^3H , and EC.

Samples for ^{18}O analysis were prepared at the University of Windsor and analyzed at the University of Ottawa. Samples for ^3H analysis were run at the Isotope Laboratory, Department of Earth Sciences, University of Waterloo. Samples from Sites 1 and 2 were analyzed for ^3H using the enriched technique which has a precision of better than 1 TU. Samples from the domestic wells were analyzed using the direct method which has a precision of about ± 8 TU. According to Egboke et al. (1983), ^3H values of less than 15 TU by the direct method almost always mean that less than 1-2 TU are actually present.

RESULTS AND DISCUSSION

We drilled 9 boreholes in the two transects. Figure 3 shows the geologic cross-section for Site 2. All of the drill sites are dominated by clayey silt to silty clay grey till interbedded with numerous sand units. Bedrock was encountered at between 31 and 34 m below ground surface. At Site 2, we encountered almost 5 m of coarse granular material above bedrock at AMA-90-4a. At wells AMA-90-5 and 6, located 31 and 68 m north of AMA-90-4a, the thick layer of coarse granular material was not encountered. We did not encounter a similar coarse granular unit at Site 1.

Although the regional map of hydraulic head in the freshwater aquifer suggests that groundwater flows from the feature outward (Cmokrak, 1991), the hydraulic head data from Sites 1 and 2 give no clear indication whether the feature is a recharge area.

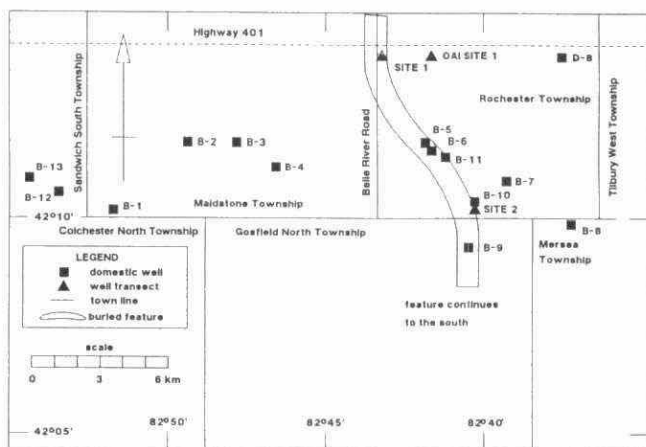


Figure 2. Locations of monitoring and domestic wells.

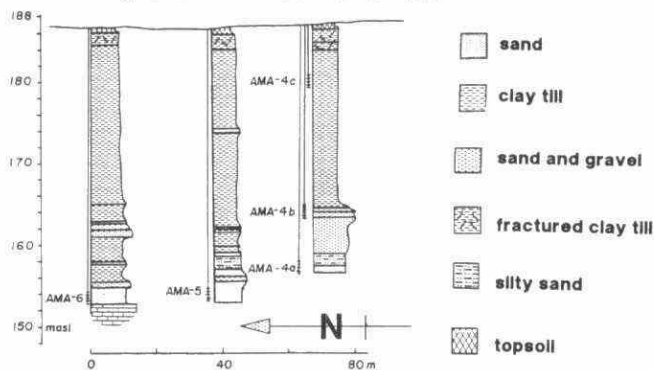


Figure 3. Geologic cross-section at Site 2.

The direction of the hydraulic gradient along the feature, from south to north, is consistent with the regional trend. From Site 2 to Site 1, the hydraulic gradient is approximately 0.0004. This is somewhat lower than regional lateral hydraulic gradients noted by Dillon (1987).

Assuming the highest observed hydraulic conductivity value (1.3×10^{-3} cm/s) and a porosity of 0.3, the groundwater velocity along the feature from Site 2 to Site 1 would be about 1.3 m/a. Although this velocity is consistent with the Dillon (1987) value, it cannot explain the presence of post-1952 water in central Essex County by lateral flow. Even if we have underestimated the velocity by a factor of 10, we could still not explain post-1952 water in central Essex County.

The vertical hydraulic gradient at Site 1 is upward (0.063), as expected, since the wells in the aquifer are flowing. This suggests discharge rather than recharge. At Site 2, the hydraulic gradient is downwards (0.003) suggesting recharge.

The preliminary isotopic and EC data (Table 1) are consistent with young groundwater in the freshwater aquifer around the feature. The more enriched $\delta^{18}\text{O}$ values that reflect more recent recharge are found near the feature. The ^3H data indicate that groundwater recharged since 1952 exists at Site 2 and at domestic wells on and near the feature. Elsewhere, the tritium data indicate pre-1952 recharge. The EC values suggest fresher water near the feature.

Studies by Desaulniers et al. (1981) and Sklash and Ibrahim (1991) have reported progressively more depleted $\delta^{18}\text{O}$ values with depth in porewater in the clayey overburden in Essex County (Figure 4). These studies conclude that the groundwater in the lower parts of the clayey aquitard were recharged under cooler climatic conditions and are several thousand years old. The $\delta^{18}\text{O}$ values in this study are almost constant with depth. The very depleted $\delta^{18}\text{O}$ values noted by others at the base of the aquitard are absent.

Our results imply much younger water in the clayey aquitard and much more rapid vertical movement of groundwater through the aquitard than reported in previous studies. The $\delta^{18}\text{O}$ distribution in the clayey overburden also suggests that the young groundwater in the aquifer is the result of vertical recharge rather than lateral arrival via the feature. The difference between the $\delta^{18}\text{O}$ distributions at Sites 1 and 2 may reflect the differences in vertical hydraulic gradient direction at the sites.

CONCLUSIONS

We used a program of drilling, hydrometric monitoring, and environmental isotope and natural chemical tracing to determine: (1) if the linear morphological feature noted by Morris (pers. comm.) in central Essex County is a buried esker, (2) if the groundwater in the freshwater aquifer around the feature is anomalously young as noted by Crnokrak (1991), and (3) to examine the spatial relationship between the young groundwater and the feature. The drilling results at Site 2 are not inconsistent with the linear morphological feature being a buried esker. The groundwater in the freshwater aquifer around the feature at Site 2 has apparently recharged within the last 40 years. Although relatively young groundwater resides near Site 1, the water is older than 40 years old. Our findings at Site 1 suggest that either the feature is much more subdued there or that we missed the feature.

Table 1. Selected chemical and isotopic data.

WELL #	DEPTH (m)	EC ($\mu\text{S}/\text{cm}$)	$\delta^{18}\text{O}$ (o/o)	T (TU)	WELL #	EC ($\mu\text{S}/\text{cm}$)	$\delta^{18}\text{O}$ (o/o)	T (TU)
SITE 1					DOMESTIC WELLS			
Well 1	31.70	1650	-12.2	<0.8	B-1	718	-13.3	<6
Well 2a	27.80	1793	-11.0	<0.8	B-2	605	-14.3	10
Well 2b	7.62	2221	-12.2	<0.8	B-3	1026	-10.7	16
Well 3	31.09	1432	-11.9	<0.8	B-4	2200	-16.1	<6
SITE 2					B-5	796	-8.3	15
Well 4a	30.48	343	-10.0	<0.8	B-6	476	-8.0	21
Well 4b	23.16	318	-7.5	2.6	B-7	465	-9.7	<6
Well 4c	7.62	563	-9.2	2.3	B-8	1530	-11.1	<6
Well 5	33.68	294	-9.6	<0.8	B-9	470	-9.7	<6
Well 6	34.14	371	-8.8	5.0	B-10	354	-9.4	14
					B-11	448	-9.6	<6
					B-12	1502	-13.0	<6
					B-13		-9.6	<6

Site 1 and 2 well tritium analyses ± 0.6 TU
Domestic well tritium analyses ± 8 TU

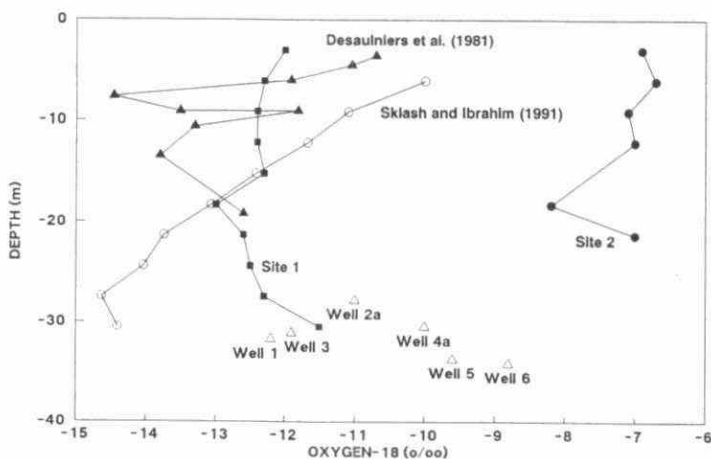


Figure 4. Vertical distribution of oxygen-18 in the clayey overburden.

The ^{18}O distributions with depth at both sites suggests more rapid vertical infiltration than at other sites studied in Essex County. These results also suggest that vertical infiltration, rather than lateral inflow, is responsible for the young groundwater in the freshwater aquifer.

We are currently trying to obtain a better spatial definition of both the feature and the young groundwater by adding to our existing transects and by adding new transects to our monitoring network. In addition, we are examining the fracture distributions and orientations in transects across the feature.

ACKNOWLEDGEMENTS

We thank Blagy Novakovic of the Ontario Ministry of the Environment and Tom Morris of the Ontario Geological Survey for their useful suggestions. The Ontario Ministry of Transport and the Township of Rochester permitted us to drill on their right-of-ways. Ontario Ministry of the Environment Research Grant Project No. 500G made this project possible. Funding from the Ontario Ministry of the Environment Environmental Youth Corps and Employment Canada SEED programs allowed us to hire additional field assistants.

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GENETICALLY ENGINEERED RESISTANCE TO POTATO VIRUS X IN FOUR
COMMERICAL POTATO CULTIVARS

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ABSTRACT

The coat protein gene from potato virus X (PVX) was introduced into four potato cultivars using a disarmed binary vector and *Agrobacterium tumefaciens* transformation system. Transgenic potato cultivars were shown to express the viral capsid protein and were resistant to PVX infection. The coat protein mediated protection resulted in a drastic reduction of the virus accumulation in transgenic potato cultivars in comparison to non-transformed cultures.

INTRODUCTION

Potato virus X (PVX) is the most widespread of potato viruses and often completely infects certain commercial stocks with yield reductions estimated up to more than 15%. PVX may be latent, without foliage symptoms or apparent effect on plant vigor except when closely compared to PVX-free stocks. Dual infections of PVX and potato virus Y (PVY) may show a synergistic increase in disease symptoms and severity known as "rugose mosaic"¹. PVX infection is systemic in the Solanaceae, usually produces local lesions in the Chenopodiaceae or Amaranthaceae and also infects certain Leguminosae. PVX is, with few exceptions, transmitted vegetatively by tubers in susceptible cultivars. Transmission through sap inoculation is accomplished by contact of plant parts in the field due to wind, animals or machinery.

PVX has many strains and the relationship among these strains has also been reported. These strains can be classified into four groups on the basis of serology and infectivity for different potato varieties². In plant cross-protection tests, strains of PVX may protect against the effects of one another completely, partially or not at all. The term of cross-protection is specifically applied to the protection of a plant by a mild strain of a virus or from subsequent infection by a virulent strain of the same virus³. The application of cross-protection in controlling plant virus diseases has been practised in some countries and has met with some successes. However, classical cross-protection is not recommended as a general practice because the so-called mild strains often reduce yield by about 5-15%, there are no appropriate mild strains of some viruses available and the practice for field use is laborious. The dominant strain of a virus may change to a non-virulent strain, and the dangers of double infections and spreading to other crops still exists⁴. However, cross-protection induced by genetic engineering overcomes these problems. Introduction and expression of foreign genes in plants with the disarmed Ti plasmid of *Agrobacterium tumefaciens* using a binary system⁵ is well documented. Tobacco plants genetically engineered to express the coat protein genes of TMV^{6,7}, Cucumber Mosaic Virus (CMV)¹⁰, PVX¹¹, Alfalfa Mosaic Virus (AIMV)^{12,13} and Tobacco Rattle Virus (TRV) can be protected against subsequent infection of these viruses.

Potato plants genetically engineered to express the coat protein genes of PVX¹⁵ and PVX and PVY¹⁶ can also be protected against subsequent infections of these viruses. The mechanisms of protection may be similar to that of classical cross protection where the uncoating of the virus is inhibited and early events in RNA replication are moderated. The plant encoded coat protein might prevent virus disassembly, recoat the RNA as it is stripped, interfere with the putative receptor site on the host, or act by some other as yet unidentified mechanisms¹⁷.

In this study the coat protein gene (CP) of PVX was cloned between the 35S promoter of cauliflower mosaic virus (CaMV) and the nopaline synthase (NOS) transcription terminator. The CP gene was then placed into a binary vector and used to transform four potato cultivars, Russet Burbank, Désirée, Shepody and Bintje. The resulting transgenic potato plants were analyzed for the expression of viral coat protein and for their resistance to PVX infection.

MATERIALS AND METHODS

Construction of recombinant plasmid of Polyrok I with sub-cloned PVX cDNA 117A

PVX cDNA clone 117A representing the 3' end of the RNA was used in this study. This cDNA clone containing the capsid protein gene was first linearized with Sall, the ends were rendered blunt by filling with

Klenow fragment of *E. coli* DNA polymerase I, and then digested with XbaI. A DNA fragment of about 1.3 kbp containing the 8K protein, the coat protein and the non-coding region was isolated. DNA fragments were then purified by 1% agarose gel electrophoresis followed by electroelution. Plasmid Polyrok I was linearized with SmaI, and then digested by XbaI. The linearized plasmid and the cDNA 117A fragment were ligated with T₄ DNA ligase (Pharmacia) and transformed into *E. coli* JM101 or HB101. Bacteria harbouring the recombinant plasmid were plated onto LB plates containing kanamycin (50 µg/ml). Polyrok I is a derivative of pBin 19³ which utilizes the trans-acting function of the vir region of the co-resident Ti plasmid in LBA 4404 to transfer sequences bordered by left and right T-DNA into nuclear genome of plants. The T region in Polyrok I contains the neomycin phosphotransferase gene (NPT II) which is driven by the nopaline synthase (NOS) promoter. The plasmid Polyrok I also contains an XbaI Sst I fragment (multiple cloning site) from pUC 19 bordered by the cauliflower mosaic virus (CaMV) promoter and a nos polyadenylation site (terminator).

Conjugation of *Agrobacterium tumefaciens* (LBA 4404 with disarmed Ti plasmid) and *E. coli* was performed in a tri-parental mating process. The donor strain *E. coli* HB 101 harbouring the recombinant plasmid Polyrok I containing the PVX coat protein gene and the helper strain, *E. coli* DH5 α containing pBRK 4013 were used in the triple mating process. Cotegrates were selected on LB plates containing kanamycin (150 µg/ml), nalidixic acid (150 µg/ml) and streptomycin (375 µg/ml).

Plant regeneration/transformation

(a) Transformation/Regeneration from tuber

Virus-free potato tubers of the following cultivars, Désirée, Russett Burbank, Shepody and Bintji were stored in the dark at 4°C. Potato tubers were washed, peeled and surface sterilized for 20 min in 1% hypochlorite solution. Tubers were then washed thoroughly in large volumes of sterile water. Discs 1-2 mm thick were sliced from cylinders of tuber tissue prepared with a cork borer.

Agrobacterium tumefaciens (LBA 4404) containing Polyrok I with PVX coat-protein was grown on selective plates. Single colonies were grown overnight in liquid LB media with 100 µg/ml kanamycin. The final bacterial concentration used was 10⁸ cells/ml. Approximately 5 µl of bacteria were applied to the surface of potato discs. On the surface of filter paper laid on MSI containing MS salts¹⁸ and vitamins with 1.5% sucrose and 0.9% bactoagar. After 48 hours of co-culture, potato discs were transferred onto selection media MS 31 containing MS salts, B5 vitamins, 3% sucrose, 1 mg/l kinetin, 1 mg/l zeatin, 1 mg/l BAP, 0.5 mg/l NAA, 100 mg/l kanamycin and 400 mg/l carbenicillin, solidified with 0.9% agar. Primary shoots that develop after 3-4 weeks were cut off and remaining discs were cultured again in the same media. Secondary shoots obtained after 2-3 subcultures were transferred to MS8 medium containing MS salts B5 vitamins, 3% sucrose, 0.5 mg/l gibberelic acid, 0.5 mg/l kinetin, 100 mg/l kanamycin, 400 mg/l carbenicillin and 0.9% agar. Rooted plants were transferred to soil and kept under polyethylene bags and high humidity for about two weeks before transferring to greenhouse conditions.

(b) Transformation/Regeneration from stem

Axenic cultures of potato cultivars were propagated *in vitro* through shoot tip cuttings and maintained in MS 8. Internodal stem explants were cut 0.5 cm in length and then cut longitudinally and then transferred to bacterial suspension for 2 min., blotted on the surface of sterile filter paper and transferred to petri dishes containing MS 1. Explants (10 per plate) were co-cultivated with *Agrobacterium* for 48 hrs., the explants were then washed in MS medium containing 1 g/L carbenicillin for 24 hrs, before being blotted dry and cultured in MS 7 medium containing B5 vitamins, 1% sucrose, 1% glucose, 1% mannitol, 1 mg/l BAP, 0.5 mg/L zeatin, 0.5 mg/l NAA, 400 mg/l carbenicillin, 100 mg/l kanamycin, solidified with 0.9% bactoagar. AgNO₃, 10 mg/l was included in MS 7 media with Russett Burbank tissue culture¹⁹. Regenerated shoots were rooted and transferred to soil as described above. All potato cultures were grown in a culture room with 22°C day, 15° night and 16 hr photoperiod with 43µEm⁻¹ sec⁻¹ light intensity.

Western blot analysis of coat protein expression

Total soluble proteins were isolated from young leaves of kanamycin resistant potato, and from healthy control plants. Leaf tissue 0.1 mg was extracted on ice with 3 ml of extraction buffer containing 0.3 M Tris-HCl, pH 8.3, 15 mM DTT, 1 mM EDTA, 1 mM phenyl methyl sulfonyl fluoride (PMSF), 1 mM Benzidin. Protein pellets were dissolved with sample buffer 0.5 M Tris-HCl, pH 6.8, 10% Glycerol, 10% SDS w/v, 5% 2- β -mercaptoethanol and 0.05% bromophenol blue and boiled at 100°C for 3 min before loading on the gel. Protein samples were run (~15 μ g protein per lane) on SDS-PAGE continuous gel (12% acrylamide for separating and 4% for stacking), cross linker 0.05% for separating and 0.1% for stacking gels in the buffer system of Laemmli²⁰, using a Bio-Rad protein II apparatus. After electrophoresis, the gel was incubated in transfer buffer (25 mM Tris HCl, pH 8.3, 192 mM glycine and 20% methanol). Polypeptides were then transferred to nitrocellulose membranes at a constant voltage of 30 V overnight. The membranes were then washed with TBS buffer (10 mM Tris HCl, pH 8 and 150 mM NaCl), and coated with 5% skimmed milk powder. Membranes were then incubated overnight with rabbit anti-PVX coat protein polyclonal antibody conjugated to alkaline phosphatase (1:1000), then carefully washed with TBS, TBS + 0.05% w/v Tween-20 and TBS incubated with the secondary antibodies (alkaline phosphatase conjugated goat anti-rabbit IgG, BioRad, U.S.A.) (1:1000). Incubation with antibodies was carried out for 2 hrs at 22°C with constant shaking. Filters were washed as described above and incubated for 10 min in developing buffer (100 mM Tris HCl, pH 9.0; 100 mM NaCl and 50 mM MgCl₂). Substrate [35 μ l nitroblue tetrazolium (NBT), and 27 μ l 5-bromo-4-chloro-3-indolylphosphate (BCIP) in 10 ml of developing buffer] was added and incubated in the dark. Colour development is stopped by washing the filters with distilled water.

RESULTS

Construction and transfer of PVX capsid protein gene into *A. tumefaciens*

PVX is the type member of the potexvirus group. The 3' end region of the genome contains a non-coding sequence of 76 nucleotides, the CP gene which encodes for a protein of 237 amino acids (26K) which is translated from a subgenomic RNA *in vivo*²¹. A large fragment of about 1.3 kbp containing the 3' end region of PVX genome, the CP and 8K protein genes was introduced into the expression vector Polyrok I between the 35S promoter and the nos terminator (fig. 1). The chimeric plant expression vector was used to transform potato cultivars by an *Agrobacterium tumefaciens* - mediated transformation system. Using this transformation system, approximately 2% of the original explants produced transformed plants which expressed detectable levels of PVX coat protein. About 20 colonies were selected from the LB agar plates containing 50 μ g/ml kanamycin. The recombinant plasmid of Polyrok I containing the 1.3 kb fragment of cDNA 117A was transformed into competent cells of *E. coli* JM 101. These colonies were labelled JP 117-1 to JP 117-20. Digestion of these colonies with XbaI and SacI indicated that JP 117-4, JP 117-9, JP 117-10 and JP 117-14 contained the 1.3 Kbp fragment of cDNA 117A. The recombinant plasmid JP 117-10 was then transformed into *E. coli* HB 101 and used for further studies. The result of Tri-parental mating of LBA 4404, *E. coli* DH 5 α and *E. coli* HB 101 (containing HP 117-10) indicated that recombinant plasmid HP 117-10 has been introduced into *Agrobacterium*. Similar results were obtained after transformation of the chimeric Polyrok I containing the PVX CP protein gene directly into *A. tumefaciens*.

Transformation and regeneration

Four different cultivars of potato, Désirée, Bintji, Russet Burbank and Shepody were used for transformation experiments. Both tuber and stem tissues were used for transformation. Primary shoots regenerated from potato tuber discs about one week after subculture into MS 31. Transformation efficiency of these shoots, although growing on 100 μ g/ml kanamycin, was lower than the secondary shoots that develop from the tubers after 2-3 subcultures in MS 31 (Table 1).

Apparently, the placement of tuber discs on auxin containing media breaks the bud dormancy and shoots emerge from already existing primordia. These shoots were unable to root in media containing 100 μ g/ml kanamycin. After the primary shoots were excised, tuber discs formed callus, and new shoots developed through *de novo* meristematic tissues within the calli.

Regeneration efficiency (number of regenerated shoots / number of explants) was higher in Desirée than in the other cultivars tested. The efficiency of regeneration is in the following order: Desirée > Shepody > Russett Burbank > Bintji. The number of shoots regenerated from each individual explant was also higher in Desirée, i.e., between 2-5 shoots developed from some tuber discs, but only one shoot / disc was retained to ensure that each of the shoots tested is a result of an independent transformation event and clonal in origin.

Efficiency of regeneration through stem explants was higher than the tuber, in both Shepody and Russett Burbank (Table 1). However, this system of transformation produced more escape shoots (non-transformed kanamycin sensitive) than tuber transformation on the basis of their ability to root and plant survival on 100 µg/ml kanamycin. During the first culture period 20-30% of the explants regenerated shoots. However, none of these shoots rooted in media containing 100 µg/ml kanamycin (Table 1). The secondary shoots regenerated through callus after removing the first shoots and successfully rooted on media containing 100 µg/ml kanamycin. Both transformation protocols used in this work resulted in morphologically normal shoots. Transgenic potato plants of all the four cultivars tested appeared normal and their morphology, growth characteristics and number of seeds were identical to their non-transformed cultivars.

Expression of PVX coat protein in transgenic plants:

The transformation of potato cultivars with the Polyrok I containing the PVX CP resulted in a total of 40 kanamycin resistant plants. All transgenic plants were analyzed by Western blotting for the expression of capsid protein. Eleven lines were found to accumulate detectable amounts of PVX CP (fig. 2). The accumulated level of CP ranged between 0.02 - 0.06% of extracted total plant proteins. Protein extracts from the four transgenic cultivars and from untransformed Russett Burbank, Shepody, Desirée and Bintji were run on SDS - polyacrylamide gel and probed with anti-PVX IgG (Fig. 2). By comparison with purified PVX CP standards, several plants showed bands corresponding to PVX CP.

Analysis of PVX CP accumulation in different leaves of transformed plants and in different plants originated from micropropagation of one original clone showed that the expression was independent of the leaf age (results not shown) and did not show any detectable somaclonal variations (e.g. clones 304 in panel A (fig. 2) are 2 different plants derived from a single clone). Furthermore, when potato seeds of the original 304 clone were grown, for the second time, and leaves were analyzed for the expression PVX CP, again measurable amounts of CP were detected.

Resistance of transgenic potato plants to PVX:

Transgenic potato cultivars and untransformed controls were challenged with 90 µg of PVX per plant. After 15 days infection under green house conditions, six grams of potato leaves were randomly picked from infected plants and used as an inoculum on the PVX local lesion host *Gamphrena globosa*. Results shown in Table 2 indicate that transgenic potato cultivars are quite resistant to PVX infection in comparison to non-transgenic controls.

DISCUSSION

In this report we describe the introduction of resistance to PVX in several economically important potato cultivars. The CP of PVX was transferred into Russett Burbank, Shepody, Desirée and Bintji. The transformation protocol used resulted in morphologically normal shoots which gave rise to transgenic plants which are visually and morphologically indistinguishable from the untransformed plants grown under the same conditions. Potato is known to be extremely sensitive to somaclonal variation in tissue culture^{22,23}. The lack of somaclonal variations in our transgenic plants is probably due to the low concentrations of growth regulators used during transformation and regeneration. Our results show that the primary shoots that regenerated from stem explants and the majority of those regenerated from tuber discs were not transgenic. Transformed shoots were recovered mostly from green callus proliferated from both stem explants and tuber discs. It is likely that cells at the cut surface of the explant pass through a period of dedifferentiation which make them permissive to transformation by agrobacterium. There are some persistent bacteria which stay on the explant after transformation. These bacteria could only be detected

if carbenicillin was omitted from the media. It appears that these resistant bacteria are those that actually achieve transformation of newly dedifferentiated cells²⁴.

PVX capsid protein was produced in detectable amounts by Western blotting in about 50% of Desirée, 35% of Shepody and 25% of each Russet Burbank and Bintji plants. This result indicates that Desirée is more amenable to transformation than the other cultivars. The lower frequency of CP expression in the kanamycin resistant plants is probably due to either expression below our detection method or to the rearrangement, deletion of T-DNA²⁵. The CP-mediated protection studies in other plant systems have reported that reduced levels of virus titer in transgenic plants relative to the corresponding non-transformed control plants⁸. We have reported that, under growth chamber conditions, transgenic potato cultivars showed much lower levels of PVX in the systemically infected leaves in comparison to the controls (Table 2). These results indicate that viral replication and/or transport of the PVX is inhibited in some way due to the presence of the viral CP. It is possible that the uncoating of the incoming virus is inhibited or the CP is competing with the virus for an attachment site.

ACKNOWLEDGEMENTS

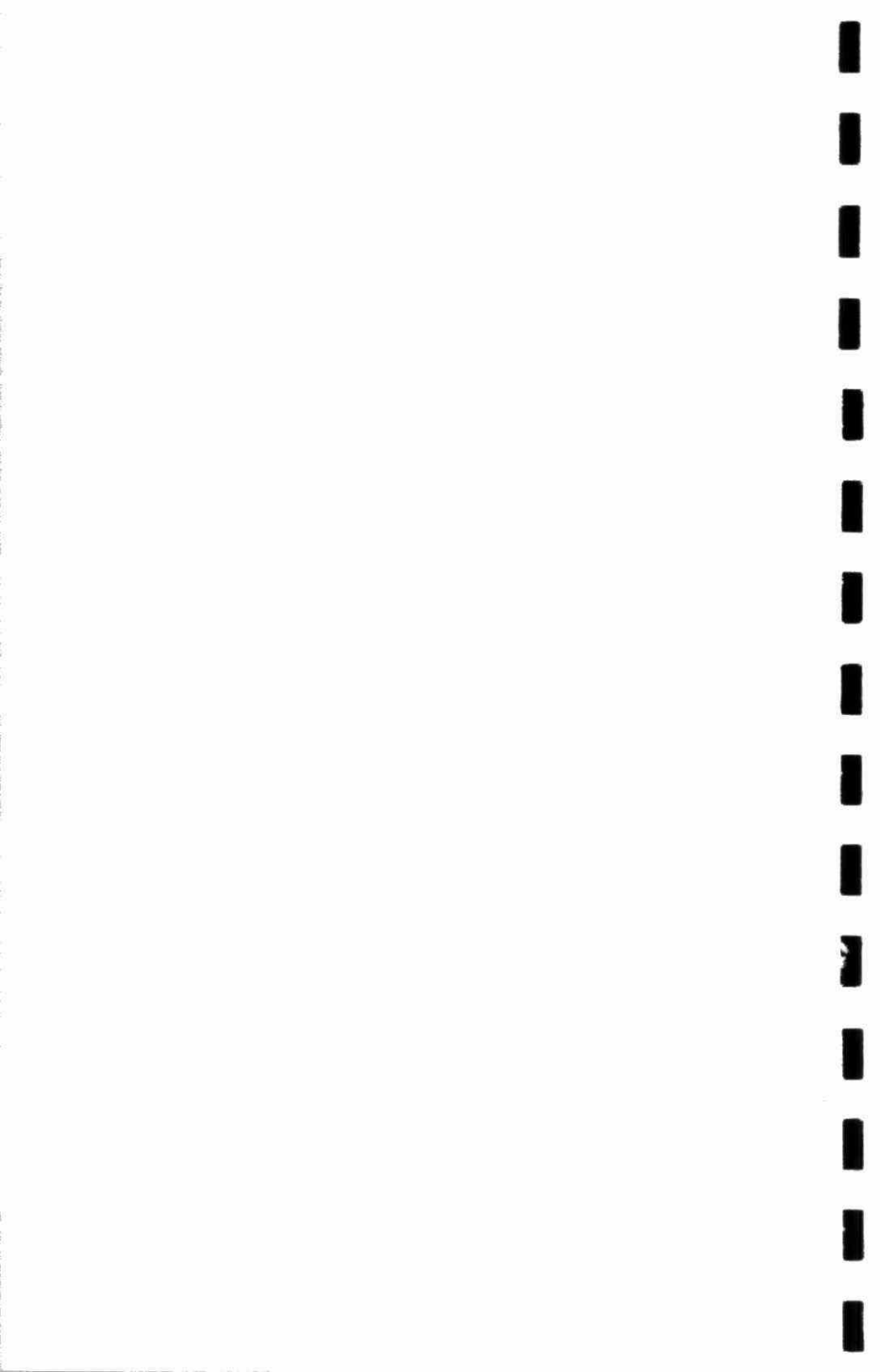
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VOLUME II
SESSION G
TECHNOLOGIES/BIOTECHNOLOGY
POSTER PRESENTATIONS



DEVELOPMENT AND VALIDATION OF A NEW, RAPID, AND ECONOMICAL SURROGATE BIOASSAY FOR INDUSTRIAL CONTAMINANTS.

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1.0 INTRODUCTION

Bioassays have been applied to numerous organisms representing different trophic levels in the aquatic food chain, including fish, arthropods, algae and bacteria. Nevertheless, the most widely-used type of procedure is the traditional acute bioassay, in which the lethality of various concentrations of a toxicant or toxicant mixture are measured within a predetermined exposure time. Standardized static bioassays, such as those using fish and crustaceans, have been and continue to be used extensively by regulatory agencies (e.g., Ontario Ministry of the Environment) in order to determine the potential effects of toxic materials. Although these tests yield quantitative measures of contamination levels, they are often either time-consuming, labour-intensive, or costly. In a review article over a decade ago, Sprague (1976) suggested that tests on lower levels of the food chain (i.e., invertebrates and algae) yield a great deal of useful data, are rapid and efficient, and, most importantly, often give results comparable with traditional bioassays using fish.

In light of these points, and the movement towards a "battery of tests" toxicological approach, there is a continuing need for the development of predictive dose-response relationships for aquatic organisms, emphasizing "lower" trophic levels. One of the main advantages of this is that organisms "lower" in the food chain may act as early warning indicators for more significant adverse effects on "higher" level food chain biota such as fish, birds, and mammals. This may be especially important where chronic toxicity is a factor.

Ciliated protists are unicellular aquatic microzooplankton which feed predominantly on bacteria and small phytoplankton. They are important organisms in the transfer and transformation of nutrients in both marine and freshwater food chains (Beaver and Crisman 1989, Pace and Orcutt Jr. 1981). Recently, with a movement in toxicological research towards (a) more rapid assessment techniques, and (b) indicators of chronic, sublethal effects of toxicants in fresh water, many investigators have turned to ciliated protists (and other aquatic microbes) as test and/or indicator organisms for the assessment of eutrophic and polluted waters, since they have been shown to be sensitive to a variety of toxicants present in the natural environment.

This study specifically addresses the development and reliability of a new protistan bioassay technique for monitoring the toxicity of pure compounds and industrial effluents. The sublethal bioassay technique utilizes the chemotactic responses of the ciliated protist, *Tetrahymena vorax*, as an indicator of aquatic toxicity.

2.0 MATERIALS AND METHODS

The approach used in this study was first to develop a protocol for a sublethal toxicity bioassay using, as an indicator, the chemotactic response of the ciliated protist, *Tetrahymena vorax*. Second, the protocol was applied to standard reference toxicants, commonly used with other standard bioassays (Environment Canada 1990). The third and final phase involved testing the protocol with toxicant mixtures (effluents) from several industries, namely, Pulp and Paper, Metal Casting, Iron and Steel, and Organic Chemical. During this final phase, two other standard MOE bioassay protocols (*Daphnia magna* 48-hr acute lethality test, Poirier et al. 1988; rainbow trout 96-hr acute lethality test, Craig et al. 1983) were also implemented using the same effluents. This allowed for comparison of the ciliate protocol to the standard assays in order to understand the relationships between the respective bioassays and to determine the utility and feasibility of the new ciliate bioassay, in light of standard test results.

Protocol Development

The goal of the first phase of the study was to establish cultures of the test organism, *Tetrahymena vorax* and to develop a working protocol (T-Maze Toxicity Assay (TMTA)) with which reference toxicants and effluents could be tested, in order to evaluate the utility and feasibility of the protocol.

Implementation of Reference Toxicant Tests

Reference toxicants were chosen in conjunction with the Ontario Ministry of the Environment. Reference toxicant tests were implemented with the TMTA, for two important reasons: (i) to determine estimates of concentrations with which tests should be run for given classes of toxicants (i.e., heavy metals, organics, etc.); and, (ii) to compare these results with those of other standard bioassays using similar reference toxicants.

The tests were carried out with four different reference toxicants, commonly used for similar purposes for other toxicity bioassays (Environment Canada 1990). The reference toxicants tested in this phase of the study were: zinc, 4-chlorophenol, sodium chloride, and cadmium. The first three reference toxicants were chosen for comparability to data from bioassays with other taxa (i.e., *Daphnia magna*, rainbow trout tests), while the fourth toxicant, cadmium, was tested for comparability to data from another ciliate protocol using chemotaxis as the test parameter (see Berk et al. 1985).

Implementation of Effluent Tests

For each effluent sample used in this phase of the study, a split sample was used to run the TMTA protocol using at least five concentrations and a control (with replication). The rest of the sample was used to carry out the standard OMOE protocols with *Daphnia magna* and rainbow trout. The TMTA test was always run concurrently with these two other tests.

3.0 RESULTS AND DISCUSSION

Protocol Development

Cultures of the test organism were established successfully and maintained throughout the duration of the study. The protocol development phase yielded positive results, as indicated by: tests with a T-maze rack, designed to eliminate biases due to gravity; clonal age tests, implemented in order to determine the age of cells in exponential phase of growth; and, control tests, designed to determine the precision and rigour of the test itself.

The aspects of the protocol requiring refinement are: (a) the treatment of cells prior to the initiation of the test; and (b) the possible use of different species as test organisms.

Reference Toxicant Tests

Tests were implemented with four reference toxicants. Using the TMTA, the only reference toxicant that yielded a significant LOEC value was sodium chloride. In this preliminary testing, it appears that the test, implemented with standard reference toxicants, is not sufficiently sensitive (in relation to other standard tests), although pre-treatment of cells through the use of a starvation buffer may yield more promising results (see Roberts and Berk 1990). Previous ciliate toxicity data using chemotaxis as the test parameter, and heavy metals as test toxicants (Berk et al. 1985) indicates that ciliates may respond sublethally in a similar range as *Daphnia* and trout do in acute lethality tests. Results from other ciliate toxicity tests are based on slightly longer response times (i.e., growth rate) or utilize direct physiological parameters (i.e., respiration) and so are not strictly comparable with this protocol, since it utilizes a test parameter that relies upon a relatively rapid behavioural response on the part of the organism.

Effluent Tests

TMTA tests were implemented with effluents from several industrial sectors. Using the test, the majority of effluents generally had little effect on the chemotactic response of the ciliates. Only 4 out of 11 effluent tests yielded results comparable to results from acute lethality tests. Interestingly, these similarities were observed with effluents from the pulp and paper sector only. It is possible, therefore, that the test may prove useful specifically for pulp and paper effluents.

There are several reasons why results from tests using whole effluents would be problematic: (1) a given effluent is made up of more than one toxicant, often acting in a complex series of relationships (that have only recently begun to be understood); and, (2) effluents are not consistent from one sampling to another due to the heterogeneity associated with effluents from different industrial processes. Since there are no comparable data published that used ciliates as test organisms, and whole effluents as test toxicants, it is difficult to evaluate the potential success of this test protocol without further study.

* * *

Results from many recent toxicological studies with ciliated protists used as test organisms have already demonstrated that these protists can be effective bioassay organisms (Berk et al. 1985, 1990; Dive and Leclerc 1975; Slabbert and Morgan 1982). Nonetheless, most of these studies used single toxicants in their evaluation of toxicity. To our knowledge, this is the first study of its kind testing whole effluents (with mixtures of potential toxicants) with ciliates as test organisms. It is not surprising, then, that there are problems and issues that arose in this study which require further resolution.

4.0 FUTURE WORK

A number of important issues for future research relating to the bioassay development emerged from the results of this study. Further work in developing this bioassay should be continued. A number important refinements to the protocol should be considered: (1) exploring variation in the preparation of cells used in the test protocol; (2) exploring reasons underlying test variability and providing some possible solutions; (3) implementing the test with other ciliate species (other than *Tetrahymena vorax*); (4) calibrating the test through an interlaboratory comparison; and (5) varying the exposure time.

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Brindle, Zheng Computer Controlled Batch Hydride Generator

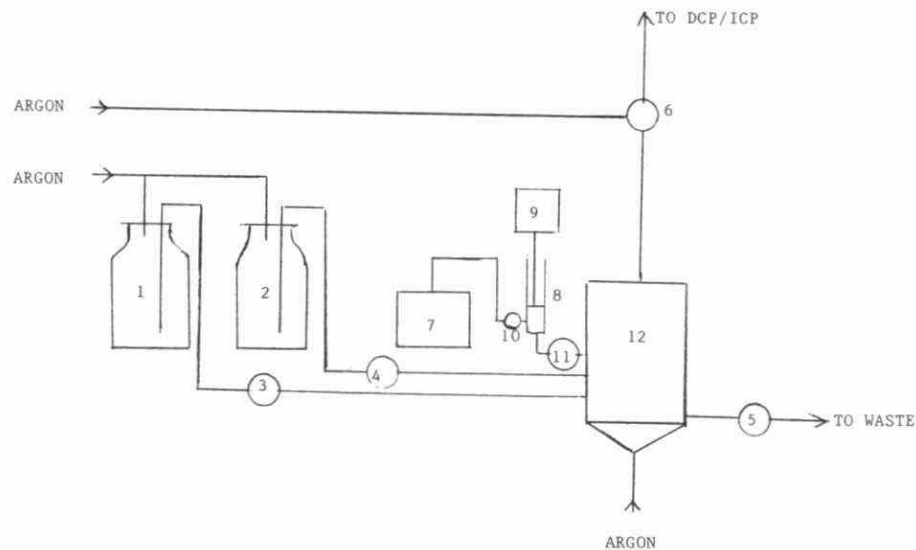
Computer - Controlled Batch Hydride Generator

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There are a number of advantages in the determination, by atomic spectroscopy, of those elements that can easily be transformed into gaseous compounds. Most important among these is the potential to transfer close to 100% of the analyte from the sample solution to the atom cell (flame or plasma). Elements that have been determined this way by forming the hydride include arsenic, antimony, bismuth, germanium, tin, lead, selenium, and tellurium. By these means, the detection limits for these elements by hydride generation is much lower, sometimes as much as two to three orders of magnitude. Although the batch process was originally the method of choice for the determination of hydride-forming elements, it has become more common to use a continuous hydride generator to eliminate the problems created by operator error in performing the various mechanical functions required in the manual batch process.

Thus the maximum potential of the hydride method has been compromised by approximately an order of magnitude in order to achieve better reproducibility. In this work we have attempted to restore the advantage of the batch system without compromising on the detection limits. The functions of the operator have been taken over by a computer which provides consistent performance in the complex series of operations of the batch system.

Samples will be placed in a Gilson 212B sampling system. Via a system of valves and a syringe, an aliquot will be delivered to the reaction vessel/gas-liquid separator. Borohydride will be delivered to the vessel from a pressurized bottle. The transient signal produced in the plasma will be captured (8000 points), a Kalman Filter programme of our own design will be used to determine the peak height and area. These will be used to calculate the concentrations in the sample. A 386 computer is required here to provide sufficient calculating speed so that the computation is not the limiting step in the sample throughput rate. The raw data will be dumped, in preparation for the next sample. The reaction vessel will be drained and rinsed with a solution from a pressurized bottle. The tubing and the syringe containing the sample residue will be rinsed and primed with the next sample.



COMPUTER CONTROLLED BATCH HYDRIDE GENERATOR

1. Rinse solution; 2. Sodium borohydride solution; 3,4,5, 10, 11. Solenoid valves;
 6. Three-way solenoid valve; 7. Gilson 212B Autosampler; 9. Syringe driver;
 12. Reaction vessel/ gas liquid separator

A New Design of an *In-situ* Separator for Continuous Hydride Generation:

Application to On-Line Pre-reduction of Arsenic(V) and
Determination of Arsenic in Water by Atomic Emission
Spectrometry

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The continuous hydride generator is one of the systems used for the spectrometric determination of hydride forming elements. In this system, the acidified sample and sodium tetrohydroborate(III) solutions are continuously pumped into a reaction coil where the analytes react with the reductant to form hydrides. The mixture of hydride and waste solution is transported into a gas-liquid separator, where the hydrides, released from the waste solution, are mixed with the carrier gas. Hydrides are carried into either the absorption cell for AAS or the plasma for AES where they are determined. Various types of gas-liquid separators, such as a U-tube separator, a

packed U-tube separator, a porous membrane separator, and a porous tube separator, have been developed. Most of these separators were designed for generation of hydrides from concentrated acid media ($[H^+] > 2M$). The hydrides are stripped from the solution by the large amount of hydrogen which is generated by the reaction between tetrahydroborate(III) and acid. If hydrides are generated from a solution with low acid concentration, the efficiency of these separators would be low, owing to the small quantity of hydrogen produced.

In our previous work, we have reported methods to generate SnH_4 and AsH_3 from low acid media (0.01-0.04 M) by means of a batch hydride generator system. In these systems, L-cysteine and L-cystine played an important role to increase of reaction rate and the sensitivity, and to decrease interference from transition metal ions, and on pre-reduction of As(V) to As(III). Compared with the continuous system, the batch system can achieve lower detection limits but it is difficult to automate and requires the operator to be well trained.

To adapt the low acid L-cysteine system to fit the continuous hydride generation system, the development of a gas-liquid separator, which works in low acid media, was necessary. Based on the idea of the hydride generator used in the batch system, an *in situ* hydride generator/separator, in which the hydrides are not only generated, but also continuously separated from the reaction solution, has been designed and tested. This paper presents the

design and characteristics of the generator and its application to the determination of arsenic.

The Design of Hydride Generator

An efficient hydride-generator should meet the following requirements: efficient mixing of the sample and reducing solutions for maximum yields of hydride; complete separation of the hydrides from the waste solutions; smooth transportation of the separated hydrides and the waste solution to the plasma and to the drain, respectively.

Based on these requirements and the chemistry of the process of hydride generation, the continuous hydride generator was designed. Sample and sodium tetrohydroborate(III) solutions are continuously pumped into the hydride generator/separator. The reactants meet and drop into the bottom of the vessel. The argon flow introduced into the generator through a glass frit at the bottom of the vessel produces fine bubbles, which not only violently mix the solution but also provide a large gas-liquid interface area to promote the hydride's being stripped from solution. To increase the total flow rate of the carrier gas, another flow of argon is introduced into the vessel from the top. The two flows of argon mix and sweep the hydrides into the plasma through the outlet port. The waste solution is pumped out to the drain. The gas/liquid separator is made wider at the top to minimize the likelihood of bubbles reaching the transfer line to the plasma.

The system has been used for the determination of arsenic, germanium, and selenium, using a direct-current plasma atomic emission spectrometer as a detector. Detection limits were found to be 4, 0.3, and 8 ng ml⁻¹ for arsenic, germanium, and selenium, respectively. In studies of interference from several of the most strongly interfering elements, the continuous hydride generator, provided superior performance over the batch process, when L-cysteine was incorporated both to reduce arsenic(V) and to reduce interferences from transition elements. Also, this technique provided significantly superior results to the conventional high acid hydride generation method.

The method has been applied to the determination of arsenic in water samples from the Ontario Ministry of the Environment. Good agreement has been found between samples analyzed by inductively coupled plasma mass spectrometry and the technique that is described.

Acknowledgement

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BASIC AND APPLIED STUDIES
WITH A
TRACE ATMOSPHERIC GAS ANALYZER
TAGA 3000

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The SCIEX TAGA series of mass spectrometers utilize an atmospheric pressure ionization (API) source. The technique of API mass spectrometry was introduced as an analytical technique by Carroll *et al.* [1]. Because of its high sensitivity towards trace compounds in air, API has since developed into a mass spectrometric technique in its own right [2]. In addition to the monitoring of trace organic compounds in ambient air, API has found analytical application as an interface for liquid chromatography/mass spectrometry (LC/MS). Our objectives have been to establish a collaborative research facility, using the TAGA 3000, at Trent University and to study the gas-phase ion/molecule reactions which occur in the API source and which may help improve the sensitivity of the instrument to a variety of compounds.

The TAGA 3000 API source is depicted schematically in Figure 1.

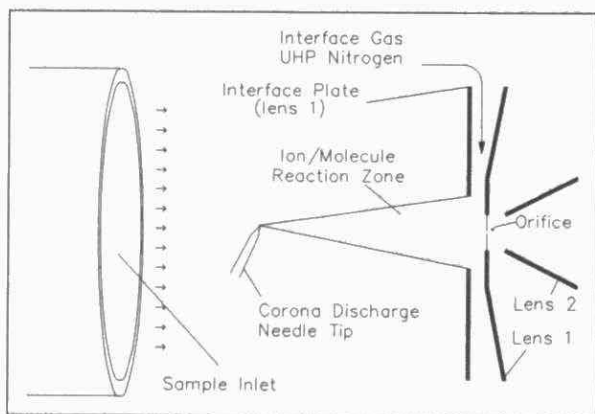


Figure 1. Schematic drawing of the API source utilised by the TAGA 3000 analyser.

The API source utilizes a high voltage corona discharge between the needle tip and the Interface Plate and draws a current of 2 μ A typically. Electron ionization of the major gases in the air stream (N_2 and O_2) initiates a sequence of ion/molecule reactions that results, in a few microseconds, in the formation of hydronium ion-water clusters ($H_3O^+(H_2O)_n$, $n=0,1,\dots$). These clusters undergo successive collisions in the source and an equilibrium is established such that, at 25°C with 5 Torr partial pressure of water (relative humidity 21%) the majority of clusters contain ca. 5-8 water molecules [3]. These hydronium ion-water clusters are the main reagent ions in API and they can protonate, through collision, molecules (B) whose gas-phase proton affinity (PA) is higher than that of the protonating cluster.

The proton transfer reaction is represented in reaction (1):



where:

A represents a gas-phase Lewis acid (proton donor)

H^+ represents the proton

B represents the target analyte (proton acceptor)

The magnitude of the enthalpy change (ΔH) associated with this reaction is referred to as the proton affinity (PA); another term which is often associated with reaction (1) is the free energy change (ΔG) or gas-phase basicity. The proton affinities of some compounds of interest are shown in Figure 2.

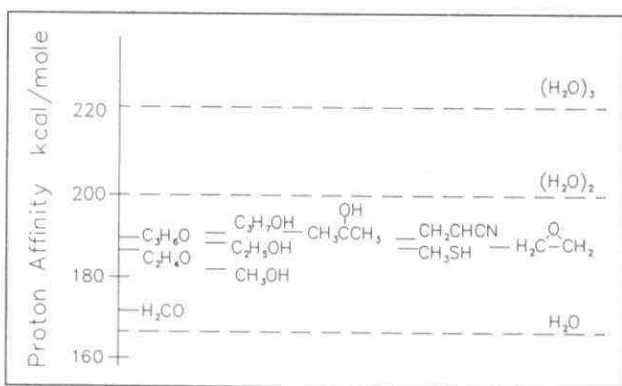


Figure 2. Proton affinities of some compounds of environmental interest.

Real-time detection of some classes of compounds in air has been a long-standing problem with the Trace Atmospheric Gas Analyser (TAGA). As the proton affinities of numerous low-molecular-weight aldehydes and alcohols are not much greater than that of water, the process of proton transfer does not occur readily in the API source of the TAGA. Furthermore, the difficulty in protonating the analyte is increased by the fact that the proton affinities of water clusters increase with increasing number of water molecules.

The specific reaction of protonation of B by water clusters is:



In the absence of hydration, proton transfer will occur at every collision for most compounds with PA higher than that of water; however, the presence of higher hydrates of water in the reagent ion composition results in a response of the instrument which may be divided broadly into three groups [4]:

- i) **K-group:** When proton transfer occurs at every collision, the system is said to be under "kinetic control" of the production of analyte molecules and results in the maximum sensitivity towards a particular compound. Generally, oxygen bases which possess a PA of greater than *ca.* 200 kcal/mole have high sensitivities which are determined by fast kinetics for proton transfer from the reagent $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions.
- ii) **T-group:** Most analytes with PA less than *ca.* 200 kcal/mole have sensitivities which are determined by the thermal equilibrium distributions of $\text{B}^+(\text{H}_2\text{O})_n$ and $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$; this condition is referred to as thermodynamic control.
- iii) **L-group:** Sulphur and carbon bases have much lower sensitivities than are expected from their PA values. This decreased sensitivity derives from the relative instability of the protonated hydrates $\text{BH}^+(\text{H}_2\text{O})_n$.

Thus a compound such as acetonitrile has a reasonably high sensitivity allowing for sub-ppb detection in ambient air, whereas another compound such as thiophene, with a similar PA, has virtually no sensitivity in the API source.

Methods for Increasing Sensitivity

1) adduct ion formation

Not only protons may be transferred to target analytes but also ions such as NH_4^+ and Cs^+ may add to target bases. We have produced Cs^+ and $\text{Cs}^+(\text{H}_2\text{O})$ ions in the TAGA 3000 airstream via a novel atomizer source, but have not yet had success in improving the sensitivities for those compounds depicted in Figure 2.

2) selective ion/molecule reactions

We have attempted to react o-methylhydroxylamine with formaldehyde in the air stream of the TAGA 3000; the signal arising from the anticipated reaction product was not intense enough to improve the sensitivity of the instrument towards this compound.

3) temperature control of the reaction zone

Kearle has reported surprising increases in sensitivity by heating the air stream. We are investigating this response of sensitivity to temperature as a means of controlling the rate of production of target analyte ions.

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Supercritical Fluid Extraction with Simultaneous Class Fractionation of PCBs and PAHs from Adsorbent Materials for Air Pollution Determinations

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Introduction

The Soxhlet leaching of adsorbent materials containing trapped organics, has traditionally been used for sample preparation for determination of trace contaminants in ambient air. This liquid extraction procedure is time consuming and create large amount of organic solvent waste. In the past few years, supercritical fluid extractions have shown excellent characteristics for rapid extraction of the organic contaminants from different matrices. In this paper, Supercritical Fluid Extraction (SFE) has been developed as an alternative to Soxhlet extraction for the determination of the trace organic contaminants in ambient air. In addition, PCBs and PAHs can be simply class fractionated when Florisil™ is used as a sorbent and by varying SFE conditions.

Experimental

The following materials were used for adsorbents in the extraction studies: Florisil™, 60/100 mesh, C18, XAD, Tenax™, Silica gel and Polyurethane Foam (PUF). Each adsorbent was cleaned by the Soxhlet method of extraction, with 250 ml of hexane for 20 hours. A standard hexane mixture was prepared containing selected PAHs and PCBs which are Acenaphthylene(ACE), 2,2',5-Trichlorobiphenyl(PCB3), 2,2',3,4,5-Pentachlorobiphenyl(PCB5), Benzo(a)anthracene(BEN), 2,2',3,4,4',5,6,6'-Octachlorobiphenyl(PCB8), Decachlorobiphenyl(PCB10), Indeno(1,2,3-cd)pyrene(IND), Coronene(COR), Benzo(g,h,i)perylene(BEP), and 2-Methyl-anthracene(Internal Standard).

SFE was performed with a high-pressure vessel which was constructed of stainless steel in the Science Machine Shop at the University of Waterloo. The vessel with a volume of 200 ml could withstand pressure in excess of 20000 psi. At the outlet of the pressure vessel, an extraction vessel consisted of two 1/4" to 1/16" reducing unions, 316 stainless steel fittings (Swagelok) and a 2.25" SS tubing. A 20 cm x 25 µm fused silica capillary was connected to the extraction vessel acting as a restrictor to maintain the pressure in the system. The capillary was directed into a 1.8-ml vial containing approximately 1.0 ml of hexane. The supercritical fluid sample was collected in the vial and concentrated to 100 µl with a gentle stream of nitrogen.

Three procedures were performed in the experiments. The first procedure was to determine which adsorbent would be the best to enable quantitative recoveries of all selected PCBs and PAHs from the matrices. Each adsorbent was spiked by injecting a small volume (100 µl) of standard mixture solution. The adsorbent in the vessel was then extracted with N₂O or N₂O with 6.5% by volume of modifier at 6000 psi for 30 minutes. The second procedure was to test the adsorbents' (XAD, Tenax™, Florisil™) ability to effectively contain trace organic contaminants during the adsorption and drying steps of the analytical process by spiking 100 µl of the standard mixture directly on top of the selected adsorbent in the extraction vessel, and passing moisture through the adsorbent for 24 h. Then the moisture was removed from the adsorbent by purging dry nitrogen through the extraction vessel for 30 minutes at 60°C. The saturated and dried adsorbent containing standard mixture was then extracted using N₂O with 6.5% by volume of modifier at 6000 psi for 30 minutes. The third procedure was to fractionate PCBs from PAHs by varying pressure of supercritical fluid N₂O.

A Varian 3500 capillary gas chromatograph, equipped with a splitless injector, a flame ionization detector (FID), and a 30 m x 0.25 µm fused silica capillary column (DB-5, J & W Scientific, California) was used for chromatographic analysis. The oven temperature was programmed to ramp from 140°C to 225°C at 15°C/min, then to 300°C at 20°C/min and held there for 14 minutes. The FID temperature was set at 325°C. The temperature of the injector was set at 300°C.

Results and Discussion

Six commercially available adsorbents are evaluated for their ability of recovering contaminants. As shown in Table 1, all PCBs could be extracted quantitatively with pure N_2O . However, PAH compounds, particularly IND and COR were poorly recovered because of strong interaction of these large compounds with the matrix.

Table 2 shows the percentage recoveries of the standard mixture from the selected adsorbents using supercritical N_2O plus 6.5% by volume (100 μ l) of methanol modifier. This method quantitatively recovered all compounds in the standard mixture from PUF, FlorisilTM, C18, Silica Gel, and TenaxTM. The difficulties in recovering high molecular weight PAHs from XAD still remain. Table 3 shows the percentage recoveries of the standard mixture from the selected adsorbents using supercritical N_2O plus 6.5% by volume of toluene modifier. Similar to Table 2, this method can also improve the recoveries of IND and COR but in a lesser extent than using methanol modifier. Quantitative recoveries of all compounds from XAD are obtainable with this conditions. This may indicate that the energy barrier of desorption between the analyte and adsorbent can be reduced by selective interaction of aromatic ring in toluene with the matrix-solute complex since XAD resins also contain aromatic rings in their structure. For other adsorbents, the method involved toluene modifier results in lower recoveries.

Apparently, if XAD is chosen to be the adsorbent, the method using toluene modifier will be superior than other modifier while methanol is suitable for the other five adsorbents.

In air sampling, the trace organic contaminants along with moisture from the air are trapped in the adsorbent matrices contained in a cartridge. The moisture must be removed from the adsorbent to ensure successful SFE and gas chromatographic analysis. Three adsorbents (XAD, TenaxTM, FlorisilTM) were investigated by comparing their

Table 1. The average recoveries for the extractions with pure N_2O at 6000 psi and 40°C

Compound	XAD	PUF	Florisil TM	C ₁₈	Silica Gel	Tenax
ACE	111.3 \pm 3	94.2 \pm 1	78.4 \pm 6	84.1 \pm 6	64.3 \pm 1	87.1 \pm 4
PCB1	105.0 \pm 3	98.5 \pm 1	99.0 \pm 10	94.6 \pm 5	88.0 \pm 2	93.9 \pm 1
PCB5	94.8 \pm 5	91.9 \pm 3	89.2 \pm 1	95.2 \pm 3	94.3 \pm 1	97.0 \pm 1
BEN	96.8 \pm 2	90.5 \pm 1	71.1 \pm 6	93.1 \pm 2	102.5 \pm 2	99.1 \pm 2
PCB8	91.6 \pm 9	92.1 \pm 1	76.9 \pm 3	84.6 \pm 4	100.5 \pm 3	97.0 \pm 2
PC10	89.3 \pm 12	92.3 \pm 1	71.0 \pm 8	71.7 \pm 15	101.9 \pm 2	97.0 \pm 2
IND	76.4 \pm 13	83.0 \pm 3	45.0 \pm 5	75.7 \pm 14	88.8 \pm 9	84.0 \pm 2
COR	12.0 \pm 8	53.3 \pm 4	2.2 \pm 1	46.2 \pm 15	35.5 \pm 4	54.7 \pm 4

Table 2. The average recoveries for the extractions with N_2O + methanol at 6000 psi and 40°C

Compound	XAD	PUF	Florisil TM	C ₁₈	Silica Gel	Tenax
ACE	109.1 \pm 13	92.3 \pm 2	86.7 \pm 3	80.0 \pm 2	112.8 \pm 19	84.4 \pm 1
PCB1	127.2 \pm 17	118.5 \pm 10	95.4 \pm 3	97.8 \pm 2	146.3 \pm 15	93.2 \pm 0.2
PCB5	102.4 \pm 6	119.8 \pm 3	91.1 \pm 2	98.7 \pm 4	126.9 \pm 8	103.9 \pm 2
BEN	64.8 \pm 19	96.8 \pm 4	139.0 \pm 2	97.9 \pm 7	135.2 \pm 4	106.3 \pm 0.3
PCB8	91.6 \pm 11	113.1 \pm 0	92.6 \pm 1	99.0 \pm 6	97.7 \pm 14	102.2 \pm 0.4
PC10	91.7 \pm 14	109.8 \pm 2	90.1 \pm 1	99.1 \pm 7	111.3 \pm 2	101.9 \pm 0.2
IND	65.4 \pm 1	100.3 \pm 11	92.6 \pm 1	100.3 \pm 13	157.5 \pm 15	92.3 \pm 1
COR	50.1 \pm 2	83.6 \pm 13	89.8 \pm 10	108.5 \pm 13	97.7 \pm 11	81.8 \pm 2

Table 3. The average recoveries for the extractions with N_2O + toluene at 6000 psi and 40°C

Compound	XAD	PUF	Florisil TM	C ₁₈	Silica Gel	Tenax
ACE	87.3 \pm 4	64.0 \pm 1	74.0 \pm 11	62.5 \pm 23	62.3 \pm 1	72.1 \pm 2
PCB1	104.8 \pm 9	93.3 \pm 4	92.3 \pm 10	78.6 \pm 10	78.9 \pm 1	90.8 \pm 4
PCB5	94.8 \pm 7	91.5 \pm 2	81.6 \pm 9	71.3 \pm 2	73.9 \pm 5	84.9 \pm 4
BEN	96.3 \pm 8	85.4 \pm 7	97.0 \pm 14	74.4 \pm 3	75.0 \pm 1	96.4 \pm 8
PCB8	101.4 \pm 11	79.3 \pm 3	76.5 \pm 8	70.1 \pm 2	75.5 \pm 5	85.3 \pm 3
PC10	107.8 \pm 14	78.6 \pm 5	80.8 \pm 9	73.6 \pm 2	78.7 \pm 5	91.1 \pm 2
IND	99.8 \pm 5	45.5 \pm 9	81.2 \pm 29	88.8 \pm 4	82.6 \pm 3	94.2 \pm 2
COR	84.6 \pm 2	68.7 \pm 4	4.6 \pm 1	81.5 \pm 1	77.6 \pm 10	76.3 \pm 9

abilities to contain trace organic contaminants during adsorption, and retain those contaminants during the drying step.

Table 4 shows the average recoveries of components from saturated with moisture and then dried adsorbents. The following observations are made: the percentage recoveries for PCBs are generally good, being independent of the nature of adsorbents; the percentage recoveries for PAHs from XAD and Florisil™ are low compared to those obtained from previous experiments (the spiked standard mixture could be extracted quantitatively using N₂O with 100 µl of modifier at 6,000 psi and 40°C). There is no significant loss of analytes during the adsorbing and drying processes when Tenax™ is used as an adsorbent.

Table 5 shows the quantitative recoveries of target contaminants are obtained when the amount of toluene or methanol is increased from 100 µl to 150 µl or 200 µl (200 µl of toluene to XAD and 150 µl of methanol to Florisil™). This method significantly improves the recoveries of IND and BEP from the saturated, dried XAD or Florisil™.

It has been found that aging of XAD spiked with 100 µl standard mixture for 24 hours at room temperature lowers the percentage recoveries of all PAHs except acenaphthylene. Table 6 shows the comparison of percentage recoveries from aged XAD with the percentage recoveries from spiked XAD (Extractions were immediately done after a standard mixture was spiked onto the adsorbent). Spiked PAHs can be extracted quantitatively from XAD using N₂O with 100 µl of toluene since thermal energy easily overcomes the low energy barriers associated with weekly adsorption between the spiked PAHs and the matrix. In the case of saturated then dried PAHs, because of the aging of PAHs for 24 hours during saturating process, the desorption is more difficult, the PAHs become strongly adsorbed onto active sites of the adsorbent. In order to extract strongly adsorbed analytes which bond to sites of XAD or Florisil™, the

Table 4. The average recoveries of contaminants from saturated and dried adsorbents

Conditions SF: N ₂ O 6,000 psi 40°C 30 min	ACE	PCB3	PCB5	BEN	PCB8	PCB10	IND	BEP
Sat., dried XAD 100 µl of Toluene	85.2 ± 9.9	92.5 ± 4.9	101.2 ± 3.5	65.8 ± 20.8	109.0 ± 2.7	109.1 ± 3.4	8.9 ± 4.7	7.7 ± 2.4
Sat., dried Tenax 100 µl of MeOH	93.0 ± 0.4	97.3 ± 4.3	101.2 ± 5.5	80.5 ± 3.8	101.7 ± 2.4	98.4 ± 3.2	89.2 ± 9.4	84.0 ± 4.3
Sat., dried Florisil 100 µl of MeOH	68.7 ± 2.7	84.1 ± 0.6	89.4 ± 4.4	79.8 ± 2.0	85.5 ± 5.6	83.9 ± 9.8	72.2 ± 13.1	68.2 ± 13.6

Table 5. The percentage recoveries of contaminants from saturated and dried adsorbents using a large amount of modifier

Conditions SF: N ₂ O 6,000 psi 40°C 30 min	ACE	PCB3	PCB5	BEN	PCB8	PCB10	IND	BEP
Sat., dried XAD 200 µl of Toluene	87.2 ± 4.5	96.3 ± 5.2	104.1 ± 7.2	108.8 ± 11.9	108.5 ± 9.4	101.8 ± 6.9	96.3 ± 7.7	86.2 ± 5.7
Sat., dried Florisil 150 µl of MeOH	71.3 ± 3.5	90.4 ± 2.7	94.3 ± 4.4	78.9 ± 6.9	108.9 ± 33.6	87.9 ± 4.4	83.6 ± 8.1	82.8 ± 6.6

Table 6. Comparison of percentage recoveries from aged XAD with spiked XAD

CONDITIONS	ACE	PCB3	PCB5	BEN	PCB9	PCB10	IND	BEP or COR
Aged XAD for 24 h 100 µl of STD N ₂ O+100 µl of Toluene 6,000 psi 30 min 40°C	89.4 ± 16.2	106.1 ± 14.8	109.1 ± 13.4	58.6 ± 12.1	103.3 ± 10.7	98.5 ± 12.9	19.0 ± 4.1	16.5 ± 4.3
Spiked XAD 100 µl of STD N ₂ O+100 µl of Toluene 6,000 psi 30 min 40°C	87.3 ± 4.0	104.8 ± 9.0	94.8 ± 7.0	96.3 ± 8.0	101.4 ± 11.0	107.8 ± 14.0	99.8 ± 5.0	84.6 ± 2.0

Table 7. Fractionation of PCBs from PAHs with Florisil™

Conditions	ACE	PCB3	PCB5	BEN	PCB8	PCB10	IND	BEP
N ₂ O 2,000 psi 25 Min 40°C	83.7 ± 1.3	92.0 ± 2.2	95.0 ± 6.7	0	119.1 ± 10.2	89.6 ± 6.0	0	0
N ₂ O/MeOH 6,000 psi 30 Min 40°C	2.2 ± 2.2	2.6 ± 2.6	10.6 ± 10.6	78.6 ± 15.2	0	0	73.8 ± 2.8	77.6 ± 0.6

amount of solvent to break the specific interaction between the target analyte and sorptive sites on the adsorbent, such as XAD, Florisil™, must be increased.

Five adsorbents were investigated to determine which would be the best adsorbent for fractionating PAHs from PCBs. The method can simplify the analysis of the complex mixtures and possibly remove the interferences from adsorbent in the air pollution studies.

Results obtained from XAD, Tenax™, C18, and Alumina show that these adsorbents cannot be used to fractionate PCBs from PAHs by varying pressure using N₂O or CO₂ since there is a large overlap between both groups. The Florisil™ is the best adsorbent providing fractionation of PCBs from PAHs. Quantitative recoveries of the PCBs were obtained from Florisil™ at 2,000 psi while 70 percentage recoveries of PAHs except ACE were extracted at 6,000 psi with modified N₂O (Table 7). This becomes evident when examining extraction pressure variation studies (Figure 1).

The PCBs are quantitatively extracted with N₂O at 2,000 psi from Florisil™. BEN is partly extracted at 3,000 psi but it is not quantitatively recovered until the pressure of the N₂O is increased to 6,000 psi. IND and BEP are quantitatively recovered only at 6,000 psi with modified N₂O.

These results tend to indicate that the fractionation of the analytes from Florisil™ is based on the classes of compounds rather than on molecular weight. For example, PCB10 has a higher molecular weight (499) than indeno (1,2,3-cd) pyrene (276), and PCB10 is extracted at 2,000 psi with other PCBs rather than PAHs. The ACE is also removed with PCBs at 2,000 psi possibly due to its lower molecular weight and boiling point.

Currently our efforts are focused on replacing nitrous oxide with carbon dioxide when modifiers are used. Initial results indicate that similar recoveries are obtained, but the extraction times are increased by about 50 %.

Conclusions

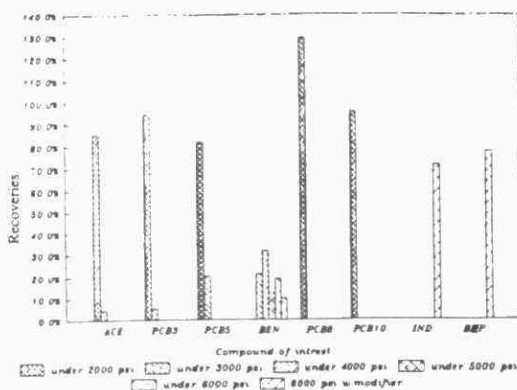
Polychlorinated biphenyls and polycyclic aromatic hydrocarbons can be quantitatively recovered from adsorbents such as XAD resin, PUF, Florisil™ and Tenax™ with SFE. SFE with methanol or toluene will result in higher percent recoveries than the extractions without modifier.

During the adsorbing and drying steps of the analytical process, no significant loss of compounds contained on all adsorbents is found. However, an aging effect was observed, which requires the addition of larger amounts of modifier to obtain full recoveries.

Fractionation of PCBs from PAHs can be performed from Florisil™ using N₂O with MeOH as a modifier by varying the extraction condition.

SFE is a better alternative to Soxhlet extraction for the determination of trace organic contaminants in the ambient air, which significantly simplifies analyses and shortens extraction time by about an order of magnitude.

Figure 1. The percent recoveries obtained by varying the pressure of the supercritical N₂O



STANDARDIZED MATERIALS AND PROCEDURES FOR HEXAGENIA, A BENTHIC BIOASSAY ORGANISM: APPLICATION TO 21-DAY SEDIMENT BIOASSAYS.

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ABSTRACT

The comparability of sediment toxicokinetic, bioassay and biomonitoring studies is often limited because neither standard control sediments nor benthic test animals are readily available. We are developing and testing contaminant-free sediments and food supplements that promote rapid growth of Hexagenia mayflies, organisms recommended for use in sediment-bioassay and ecotoxicological studies. We have reared larvae from egg to maturity in as little as 3 mo.

Laboratory cultured animals and sediments were used in a serial dilution sediment bioassay of sediment highly contaminated with organochlorine compounds from the Detroit River (Trenton Channel). Large larvae were subjected to 21-d exposures of a sediment dilution series (1:0, 1:1, 1:3, 1:7, 1:15 or 0:1 mixture of Trenton Channel and synthetic sediment). Larval size was ascertained before and after exposure by measuring photographic images of individuals.

We detected no significant effect of the contaminated sediment on either survival or growth of Hexagenia. Variability among replicates and relative insensitivity of mature larvae to contaminants both contributed to this result.

Gas chromatographic analysis of contaminants in sediments and animals indicated significant compound-specific variability in dilution ratios and patterns of uptake. Ratios of concentrations of few compounds matched the nominal values expected as a result of dilution. Ratios for pentachlorobenzene (QCB) and hexachlorobenzene (HCB) greatly exceeded the 16-fold difference expected in the 15:1 sediment mixture. Ratios for PCBs and other compounds ranged from 3-12. Different carbon species possibly influence binding capacity of the sediments. Uptake of QCB and HCB by Hexagenia decreased log-linearly among sediment dilutions, whereas levels of PCBs and other compounds were greatest in animals reared at intermediate (1:1 dilution) concentrations. Bioaccumulation factors varied greatly among sediment dilutions (6-20 in 1:15 dilution vs. 1.5-2 in 1:0 dilution). Feeding/metabolic inhibition or differential contaminant binding to varying carbon species are possible explanations.

Previously proposed sediment bioassay guidelines recommend control vs. treatment comparison of 21-d growth and survival of Hexagenia larvae, (10 half-grown individuals/2 L container, n=3 containers/treatment). However, such tests have limited statistical power: true differences between means $\geq 50\%$ and coefficients of variation $\leq 20\%$ are required for treatment effects to be judged significant at $p < 0.05$ in 80% of experiments. At least 5 replicates should be used in 2-sample sediment bioassays.

A serial dilution bioassay permits evaluation of relative toxicity of bulk sediments analogous to procedures used for water bioassays. Additionally, gas chromatographic analysis of larvae and sediments from various dilutions can provide important information on variation of bioaccumulation factors with respect to sediment contaminant levels.

1. INTRODUCTION

Various bioassay and biomonitoring techniques assume that variation in test organism survival, growth and/or ultimate contaminant burden directly reflect the concentration and toxicity of contaminants in the sediment. Response differences between contaminated and uncontaminated site-collected treatments is assumed to reflect the influence of the contaminants alone. We have proposed that comparing both field-collected reference and contaminant-influenced treatments with a standard, laboratory-prepared control permits one to ascertain those test organisms' responses to on-site sediments that result from inappropriate or suboptimal physical sediment characteristics (Ciborowski and Corkum 1989).

We have been evaluating the utility of synthetic sediment as a reference (control) substrate in short- and long-term sediment bioassay procedures using *Hexagenia* mayfly larvae. *Hexagenia* larvae are important field and laboratory test organisms that have been recommended as a standard for evaluation of toxic effects of contaminated sediments (International Joint Commission 1987, Lomas and Krantzberg 1988). In this paper we report on; a) 21-day larval growth and survival; b) 21-day patterns of contaminant transfer; and c) the statistical power of bioassays that employ various levels of replication.

2. METHODS

2.1. Sediment Collection and Preparation

2.1.1. Contaminated Sediment

In June 1991, surficial sediment (top 7.5-10 cm; water depth 1-1.5 m) was collected with a metal bucket from the Trenton Channel of the Detroit River (42°15'N, 83°10'W; site 107 of Furlong et al. (1988)). The sediment was stored refrigerated in 8-L hexane-rinsed galvanized buckets until required. Sediments from this area are highly contaminated with organochlorine compounds (Thornley and Hamdy 1984; Furlong et al. 1988).

2.1.2. Synthetic (STND) Sediment

Our synthetic standard reference sediment (STND) consisted of a 21:21:16 ratio (dry mass) of silica sand (180-500 μ m diameter), prepared clay (Lewiscraft Sculptor's Clay, Lewiscraft Ltd., Toronto, ON, M1S 2S2), and potting soil (Zehr's No Name Potting Soil, Zehr's Ltd., Toronto, ON). The components were combined and dried prior to use according to procedures described by Hanes et al. (1990). The particle size distribution and organic content of this mixture are especially suitable for culture and growth of *Hexagenia* larvae. We have reared larvae from egg to maturity in this material in as little as 3 mo.

2.1.3. Experimental Bioassay Sediments

Dilutions of contaminated sediment were prepared by combining known proportions (by dry mass) of Trenton Channel sediment (loss on ignition (LOI) 10.6%) with STND sediment (LOI 8.4%). In July 1991, we produced 12 L of each of 6 mixture, designated 1X, 0.5X, 0.25X, 0.125X, 0.063X and 0X, where 1X represented 100% Trenton Channel sediment and 0X represented 0% Trenton Channel sediment (= 100% STND sediment). These corresponded to Trenton:STND ratios of 1:0, 1:1, 1:3, 1:7, 1:15 and 0:1, respectively.

Diluted sediments were prepared by measuring a required quantity of naturally wet Trenton Channel sediment into a container, stirring in STND sediment in dry form (<5% moisture), adding enough dechlorinated tapwater to produce a sediment of moisture content equivalent to 1X sediment, and mixing thoroughly. All sediments were prepared on 5 July 1991.

2.2. Experimental Animals

The 21-day bioassay study was conducted with 120-day-old larvae (mean head widths among replicates 1.95-2.32 mm; mean body lengths 17.4-19.9 mm). Larvae had been hatched from eggs in the laboratory and mass-reared in STND-1 sediment on a diet of Tetramin^R (Hanes et al 1990). On the first day of the study larvae were placed randomly in water-filled petri dishes (5 larvae per dish) whose bottoms had been marked with 1-cm lines. Each petri dish was photographed and the larvae were then transferred to a bioassay jar (10 larvae per jar) as required.

Jars were maintained, aerated for 21 d. Larvae were fed twice weekly with a 2 mL of a mixture of Tetramin^R, baker's yeast and alfalfa powder (Hanes et al. 1990) to minimize effects of potential contribution of differences in edible organic matter to larval size/survivorship differences at the end of the experiment.

2.3. Bioassay Experiments

2.3.1. Experimental Procedures

The experiment was initiated in July 1991 following recommendations of Lomas and Krantzberg (1988) for 21-day *in situ* sediment assessment. Five replicate sediment samples of each treatment were prepared and placed within 2-L Universal glass jars (5 jars x 6 sediment types = 30 preparations). An equal volume of material (350 mL) was added to each jar to produce a sediment depth of 2 cm. Because Trenton Channel and STND sediments differed in density, wet masses differed slightly among treatments. Thirty identical preparations were made simultaneously for a long-term study (discussed elsewhere).

Following addition of sediment, dechlorinated tapwater was placed in each jar and lids were loosely put into place. Capillary tubing was inserted through the lid of each jar and below the water surface to provide aeration. Jars were kept at room temperature (20-22°C). Measurements of conductivity, pH and dissolved oxygen concentration indicated that all replicates had equivalent water chemistry at the beginning and end of the experiment (pH = 6.7, conductivity 390 $\mu\text{S cm}^{-1}$, oxygen saturated).

The experiment was terminated on 14 August 1991. We removed larvae while attempting to minimize disturbance to the sediments. Surface water was carefully poured off and replaced with carbonated water (club soda), which anaesthetizes larvae and causes them to rise to the water surface. All larvae from a jar were removed, placed into a gridded petri dish and photographed. Larvae were subsequently frozen and stored in hexane-rinsed jars prior to gas chromatographic (GC) analysis.

Aliquots of sediment from three randomly selected replicates of each of the sediments were placed in hexane-rinsed amber jars and stored frozen. Only the 0X, 0.063X and 1X sediment analyses have been completed to date and are reported herein.

2.3.2. Determination of Larval Growth

The size of each larva at the beginning and end of the bioassay study was determined by measuring its photographic image (head width and body length) with reference to the size of the image of lines on the bottom of petri dishes in which the larvae were photographed. Head width provided less within-jar variation than body length. Thus, this variable was used to estimate larval growth. Growth was defined as the instantaneous percentage change in mean larval head width ($100 \times \ln(\text{ratio of final: initial head width})$) estimated over the course of the experiment. Larval growth has been measured for only the 1X, 0.063X and 0X treatments. Only these data are reported here.

2.4. Contaminant Analysis

Triplicate sediment samples and duplicate *Hexagenia* samples were analysed for organic contaminants concentration in the Great Lakes Institute analytical laboratory. Twenty-five-g aliquots of sediment were Soxhlet extracted for 24 h and analyzed according to the procedures described by Gobas et al. (1989). *Hexagenia* larvae (1.0-1.5 g wet mass composite from one bioassay jar) were homogenized with mortar and pestle and extracted by solid-liquid column extraction using 20 g anhydrous sodium sulphate and 300 mL 50% dichloromethane-50% hexane mixture as the solvent (Kovats and Ciborowski 1989).

Extracted samples were injected into a Hewlett-Packard model 5790A GC equipped with a 25 m x 0.25 mm fused silica column and an electron capture detector. Concentrations of 19 contaminants (14 PCB congeners, total PCBs, DDE, QCB, OCS, HCB, trans-nonachlor) were quantified based on peak patterns and comparison to those in standard mixes of known concentrations.

3. RESULTS AND DISCUSSION

3.1. *Hexagenia* Growth and Survival

Little mortality occurred over the 21-day interval of the study (Fig. 1A). One hundred percent survival was observed in at least two replicates of each treatment. Mean (\pm SE)

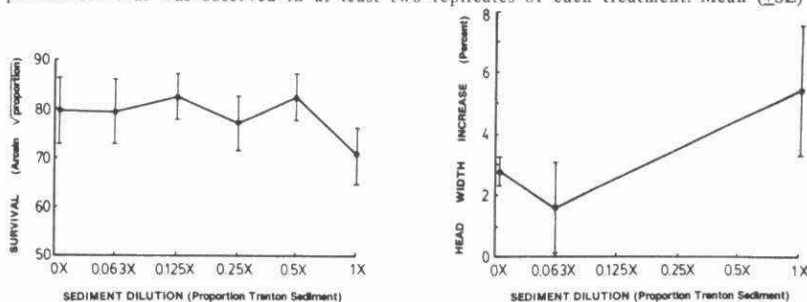


Figure 1. Mean (\pm 1 S.E., $n=5$) survivorship and growth in head width of *Hexagenia* larvae after 21 days in mixtures of sediment ranging from pure (1X) Trenton Channel sediment to pure (0X) STND sediment. **Left.** Survivorship (arcsin square root of proportion). **Right.** Percentage growth ($100 \times \ln(\text{final head width}/\text{initial head width})$). Data for intermediate dilutions presently unavailable.

Table 1. Mean (SE) concentrations of contaminants in sediments (left column, n=3) and *Hexagenia* (3rd column, n=2) from 0.063X dilution treatment, ratios of concentrations in 1X:0.063X treatments of sediment (2nd column) and *Hexagenia* (4th column), and *Hexagenia* bioaccumulation factors in 0.063X (5th column) and 1X (6th column) treatments.

Compound	Concentration in 0.063X Sediment (ug/kg carbon)	Ratio of Sediment Concentrations (1X/0.063X)	Concentration in 0.063X <i>Hexagenia</i> (ug/kg Lipid)	Ratio of <i>Hexagenia</i> Concentrations (1X/0.063X)	Ratio of HEX.:SED. Concentrations (0.063X)	Ratio of HEX.:SED. Concentrations (1.0X)
GCB	11.0 (3.6)	22.40	155.7 (10.3)	5.55	14.15	3.51
HCB	70.4 (19.2)	30.14	939.3 (119.1)	7.13	13.34	3.16
OCS	27.1 (2.5)	14.94	187.7 (16.4)	1.71	6.92	0.79
Trans-nonachlor	1.8 (0.9)	12.39	51.6 (2.2)	1.17	28.68	2.72
p,p'DDE	121.7 (8.0)	11.86	1786.0 (134.3)	1.91	14.67	2.37
PCB 28	34.4 (9.0)	3.41	217.0 (17.0)	2.29	6.31	4.25
PCB 52	104.8 (4.6)	5.66	1405.4 (87.5)	1.71	13.41	4.05
PCBs 66/93	129.2 (5.5)	6.21	1558.2 (230.6)	1.57	12.06	3.06
PCB 87	37.5 (1.2)	8.12	512.2 (38.6)	1.59	13.65	2.67
PCB 99	36.3 (0.7)	6.98	641.1 (63.5)	1.23	17.66	3.12
PCB 101	74.7 (2.1)	7.61	1162.5 (82.7)	1.41	15.56	2.88
PCB 110	66.8 (4.7)	7.75	859.8 (70.1)	1.72	12.88	2.85
PCB 118	66.1 (0.5)	7.85	924.7 (123.5)	1.45	13.99	2.58
PCB 138	107.6 (25.0)	7.75	956.6 (91.1)	1.40	8.89	1.6
PCB 153	44.3 (2.3)	10.59	678.7 (38.5)	1.21	15.31	1.75
PCBs 170/90	23.3 (4.3)	6.66	177.9 (13.0)	1.46	7.64	1.67
PCB 180	34.9 (0.3)	10.84	378.5 (7.8)	1.18	10.84	1.18
PCBs 182/87	20.6 (1.0)	11.56	211.7 (23.8)	1.37	10.30	1.22
PCB 194	6.6 (3.4)	15.78	133.2 (60.8)	1.20	20.33	1.54
Total PCBs	1468.5 (341.6)	7.83	13060.6 (1244.5)	1.40	8.89	1.58

among-replicate survival was lower in the 1X sediment treatment (86±5.1%, n=5) than in any of the other treatments (95-98%) but the difference was not significant (1-way ANOVA of arcsin square root transformed data, $p > 0.05$). Coefficients of variation (V) ranged from 11.9 to 18.7 percent.

Larval growth ranged from a mean loss of 3% of head width to an increase of 11% within individual jars over the duration of the experiment. Sexual dimorphism of larger larvae and an approach to emergence with consequent distortion of body dimensions contributed to size variability. There were no significant differences in mean growth among the 0X, 0.063X and 1X treatments, although growth appeared to be stimulated by pure Trenton Channel sediment (Fig. 1B; $p > 0.05$, Kruskal-Wallis test; coefficient of variation of Log transformed data ranged from 9 - 80%).

3.2. Contaminant Uptake

3.2.1. Sediment Contaminant Concentrations

Organic content of the Trenton Channel and STND sediments were comparable (10.6 and 8.4% at the beginning of bioassay). Food addition during the experiment increased organic content of the STND sediment by approximately 1.8 percentage points but did not influence organic content of the Trenton sediment.

Although the dilution of Trenton Channel sediment with STND sediment was expected to produce a proportional change in contaminant concentrations (16-fold between 1X and

0.063X), this did not appear to occur. The 1X:0.063X ratio of concentrations varied with individual compounds (Table 1). Pentachlorobenzene (QCB), hexachlorobenzene (HCB) and octachlorostyrene (OCS) ratios met or markedly exceeded expectations. Ratios for the remaining compounds were all <16. Ratios for PCBs ranged from 4-16 and tended to increase with increasing congener number. Because organic carbon content of STND and Trenton Channel sediments were very similar, ratios based on dry mass were almost identical to ratios based on carbon mass of the sediments.

We speculate that the noncongruence of ratios of individual contaminants between sediments may relate to qualitative differences in the organic carbon fraction of the two sediment types. We believe that the carbon species of STND sediment bound QCB and related compounds less tightly than the carbon species associated with Trenton Channel sediment. The contaminant dynamics only partly parallel patterns of contaminant hydrophobicity. Although values of the relatively soluble nonPCB compounds exceeded the expected 16-fold dilution ratio, ratios progressively increased with decreasing solubilities of the various PCB congeners.

3.2.2. Contaminant Uptake by Larvae

Larvae reared in reference (0X) sediment had very low but detectable concentrations of all contaminants assayed except OCS. Concentrations of QCB, HCB, OCS and trans-nonachlor were all <1.0 ug/kg wet mass. Concentrations of p,p'-DDE and 14 PCB congeners were all <2 ug/kg wet mass. Mean (\pm SE) total PCB concentration (expressed as Aroclor 1254/1260) was $21 \pm (4.3, n=2)$ ug/kg wet mass. These concentrations are equivalent to or less than contaminant levels reported for larval and adult *Hexagenia* collected from 'uncontaminated' Ontario reference field sites (e.g., Bedard 1990, Kovats and Ciborowski 1989).

Patterns in contaminant uptake by *Hexagenia* with respect to specific contaminants paralleled the differences in concentration ratios between 1X and 0.063X sediment dilutions. Concentrations of QCB and HCB in *Hexagenia* increased approximately log-linearly with increasing proportion of Trenton Channel sediment (Fig. 2A). This pattern was evident for contaminant concentrations expressed both on a wet mass and a lipid mass basis, although lipid mass expression produced less variation among repli-

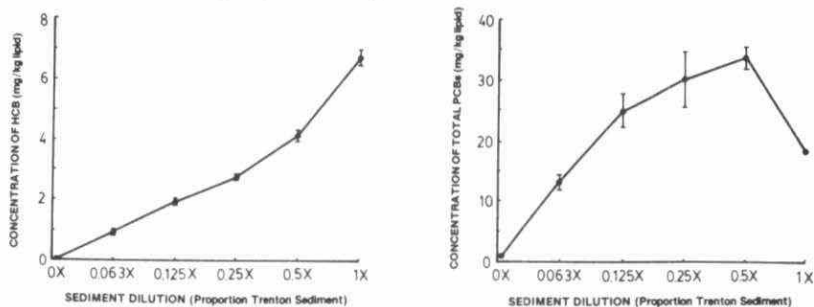


Figure 2. Mean (\pm 1 S.E., n=2) concentration of organic contaminants in *Hexagenia* larvae after 21 days in mixtures of sediment ranging from pure (1X) Trenton Channel sediment to pure (0X) STND sediment. Left. Concentration of hexachlorobenzene (mg/kg lipid). Right. Concentration of total PCBs (mg/kg lipid).

cates. Lipid comprised 1.3-2% of larval wet mass. Regression analysis revealed a significant positive relationship between *Hexagenia* concentration, of QCB and HCB and $\ln(\text{Proportion of Trenton Channel sediment})$ ($F=255$, $p<0.001$, $R^2=0.94$). However, there was significant deviation from linearity ($p < 0.005$). Concentration of PCBs increased log-linearly with increasing proportions of Trenton Channel sediment for all dilutions except the 1X treatment (Fig. 2B; linear regression analysis, $F = 24.45$, $p<0.05$, $R^2=0.84$). These findings are consistent with a differential uptake model of bioconcentration and bioaccumulation. Relatively soluble compounds ($\text{Log } K_{ow}<6.0$) are thought to be taken up primarily by transport across respiratory surfaces, whereas ingestion pathways are more important for the less soluble compounds (Landrum and Poore 1988, Bedard 1990). We suspect that the reduction in PCB uptake in 1X treatments reflects a change in either feeding or physiological/metabolic behaviour of larvae. However, no significant differences in growth were found among treatments, so that these differences cannot be attributed to feeding rate per se. Accordingly, differential binding of contaminants to the carbon species present in Trenton Channel sediment is a more tenable explanation for these differences.

Variation in bioaccumulation ratios among sediment dilutions (Table 1) are also consistent with a hypotheses of reduced feeding or differential contaminant binding. Bioaccumulation factors (BAF) ranged from 6 - 30 (median 13.3) in 0.063X sediments but from only 0.8 - 4.3 (median 2.6) in 1X sediment. A decrease in BAF with increasing hydrophobicity among PCBs may indicate differences in the time required for the compounds to achieve equilibrium among sediment, water and mayfly larvae.

Although our bioassay failed to show significant mortality or growth effects of contamination on *Hexagenia*, contaminant transfer is obviously significant. Noncongruence in the behaviour of different contaminants at various dilutions has important implications for the evaluation and interpretation of bioassay results. There is clearly an interaction between contaminant characteristics and mode of uptake by larvae. The ultimate effect on BAFs and the organisms' success requires further study.

3.3. Statistical Power of Sediment Bioassays

Previously proposed sediment bioassay guidelines (Lomas and Krantzberg 1988) advocate comparison of 21-day growth and survival of *Hexagenia* larvae in triplicate samples of contaminated sediments to field-collected reference sediments. The ability to detect a significant effect (statistical power) depends on three factors: the true magnitude of the effect (D the difference between reference and treatment), among-replicate variability within treatments (expressed by the coefficient of variation V [$100 \times \text{standard deviation}/\text{mean}$]) and the number of replicates per treatment (n). The power of a bioassay test is proportional to $(n \cdot D)/V$. If one wishes to be reasonably certain of detecting a specified difference D , (ideally defined by predetermined guidelines) then replication levels must be selected to accommodate the amount of among-replicate variability of the data.

Table 2 outlines the degree of replication necessary to provide 80% certainty of detecting a difference between two means (significant at $p < 0.05$) for different values of V and D . A bioassay test employing triplicate samples would be 80% certain to show a difference between two means of 50% if V was 20% or less (boldfaced entry of Table 2). Among-replicate variation in size and survival is often 30-50% or more for laboratory cultures of aquatic invertebrates such as *Hexagenia* (Hanes and Ciborowski 1991). Clearly, designs that employ triplicate samples of treatments are relatively insensitive. With 40% variation, growth or survival in controls must be almost half or double those in a treatment if an effect is to be judged significant in 80% of tests. We recommend employing a minimum of 5-6 replicates if differences as small as

Table 2. Minimum sample size necessary to be 80% certain that a specified true difference between two groups will be found significant ($p < 0.05$). V is the within-replicate coefficient of variation ($(s/\bar{X}) \times 100$). Line transecting table indicates minimum differences likely to be designated significant with triplicate sampling. Entries for which $n > 100$ may be overestimated by approximately 2%. Three replicates are considered an absolute minimum sample size if outliers are to be identified. Entries were determined from power formulae given by Sokal and Rohlf (1981).

V(%)	TRUE DIFFERENCE BETWEEN CONTROL AND TREATMENT MEANS (%)									
	100	90	80	70	60	50	40	30	20	10
100	14	18	22	29	39	54	67	99	201	801
90	12	14	18	23	32	41	55	73	163	649
80	9	11	14	18	25	36	47	67	129	513
70	7	9	11	14	19	28	40	55	99	393
60	5	7	8	11	14	20	32	45	81	289
50	4	5	6	7	10	14	22	36	67	201
40	3	3	4	5	7	9	14	25	52	129
30	3	3	3	3	4	5	8	14	32	73
20	3	3	3	3	3	3	4	7	14	33
10	3	3	3	3	3	3	3	3	4	14

50% are to be judged significant (10-12 replicates x treatments in a two-sample study).

4. PROVISIONAL CONCLUSIONS

Simple sediment bioassays involving triplicate replicates of reference and treatment sediments are unlikely to have the power necessary to indicate toxicity except in extreme cases, especially if large, relatively tolerant mature animals are used. Replicated regression analyses are less powerful than two-sample comparisons if a fixed total number of observations is to be employed (Sokal and Rohlf 1981). However, designs such as the serial dilution protocol can provide important information regarding the dynamics of contaminant uptake. We recommend increased levels of overall replication be employed in sediment bioassays (a minimum of 10 observations among all groups/replicates), regardless of the design to be employed.

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DIAGNOSTIC EXPERT SYSTEMS: ENCODING CHEMICAL KNOWLEDGE IN AAdiagnosis

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Abstract This paper describes the design and implementation of a diagnostic expert system that is used to diagnose problems in the analysis for metals by atomic absorption spectrometry. In the AAexpert system, problems associated with instrumental analysis are classified into (a) problems that are detected during instrument optimization and (b) problems that are chemical in nature and are encountered only during sample analysis. The chemical knowledge is encoded at three different levels, namely (a) the primary level, (b) the secondary level, and (c) the tertiary level. A series of interconnecting rules are generated on compiling the secondary level of knowledge. The method of encoding chemical knowledge at three levels provides a robust technique which is generally applicable to encoding chemical knowledge for use in advisory expert systems.

ENCODING CHEMICAL KNOWLEDGE FOR AAdiagnosis

Our development project is part of a large, multi-component expert system named ACexpert, a system which is concerned with all aspects of chemical instrumental analysis (1). The expert system shell KDS 3 (from KDS Corporation, 934 Hunter Road, Wilmette, IL 60091, USA) has been chosen as the shell within which our expert system will be developed. The role of AAdiagnosis in AAexpert is to provide advice to users in areas related to diagnosing problems associated with the measurement of data. Through experiment, a series of unique symptoms that degrade the analytical quality of data obtained by flame atomic absorption spectrometry (AAS) have been identified (Table 1). Table 1 lists the symptoms (conditions) encoded in AAdiagnosis. In some cases the symptom can only be observed under special conditions. For example, when a sample is aspirated into the flame the real time signal from the photomultiplier tube exhibits a characteristic shape and noise when plotted as a function of elapsed time. Deviation from this signature can be used as a sensitive diagnostic tool.

We have evaluated the symptoms reported earlier (2, 3) and have adjusted the entries in the knowledge base to include additional symptoms associated with measurement of data by flame atomic absorption spectrometry. We find that analysis of information regarding noisy data requires certain instrumental set up and sample property information. These conditions have been incorporated into the knowledge base. The major causes of these symptoms have been deduced. The knowledge base of symptoms and causes and the resultant rule base is general for all flame AAS analyses and so these data can be applied to other systems.

In our approach we connect the instrumental set up conditions and sample chemical properties, to the analytical symptoms through logical relationships established at the primary and secondary level of knowledge development. The most important feature of any expert system is the construction of the knowledge base. Since the KDS knowledge base is represented as a matrix of facts we can build up information in three stages. The requirements to "fill in" the matrix leads in encoding the chemical information at three different levels, namely (a) the primary level, (b) the secondary level, and (c) the tertiary level (4). At the primary level, a condition is added to the knowledge base in order to discriminate between the existing knowledge. At the secondary level, the logical connections between conditions and case conclusions not related at the primary level are connected logically. Thus knowledge is represented in the form of a matrix of facts in which columns are represented by case conclusions and rows are represented by conditions. Table 2 shows the matrix of knowledge in AAdiagnosis. The knowledge in the matrix is said to be completely "filled" when the developer does not leave any condition unrelated with conclusions. The final level of expertise is added to the module when the developer encodes different combinations of symptoms that result in causes that exist in the knowledge base. These parallel cases added to the module depend on the previous experience of the expert.

DISCUSSION

Rule based expert systems have been widely adopted as a method of representing knowledge diagnostic expert systems. One of the best understood rule based expert systems in the field of analytical chemistry is the program for

interpretation of infra red spectra (PAIRS) by Woodruff and Smith (5). This program was designed to aid the chemist in interpreting infra red spectra of liquid and solid samples. Changing or adding rules was not difficult but the drawback of the system was expressing chemical knowledge as well defined rules.

We have described a method of encoding chemical information for problems encountered during metal analysis by flame atomic absorption spectrometry. The constraints involved in adding new knowledge to an expert system based on conventional language led us to use of an expert system shell for the development of AAdiagnosis. We chose a rule based expert system shell that requires the chemical knowledge to be encoded in the form of case histories. AAdiagnosis treats the symptoms and the related causes as data. The primary level of knowledge relates symptoms directly with causes. The secondary level of knowledge relates symptoms with symptoms. The rules in AAdiagnosis are generated after compiling the secondary level of knowledge and therefore relate symptoms. The symptoms are universal and together with the causes represent our experimental results.

ACKNOWLEDGEMENTS

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Table 1. List of symptoms (conditions) in AAdiagnosis

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1. Relative standard deviation of the measurements are high(>5%) and absorbance values are not precise.
 2. Absorbance values are 50–80% of the expected values in the calibration graph.
 3. Absorbance values are 20–40% of the expected values in the calibration graph.
 4. Noisy absorption signal (observed from signal graphics).
 5. Occasional pulse up or down in signal (observed from signal graphics).
 6. Signal decays during absorption (observed from signal graphics).
 7. Pulsating signal (observed from signal graphics).
 8. The calibration graph levels off at high concentrations.
 9. The calibration curve is sigmoidal.
 10. There is a change in absorption sensitivity between re-calibration.
 11. Drift in baseline.
 12. The flame has a ragged appearance.
 13. Bubbles form in the capillary tube.
-

Instrumental conditions that relate directly to the diagnostic rules:

14. Integration time is more than 3 seconds.
 15. An oxidizing flame is being used.
 16. Pressure is higher than 70 psi in the fuel cylinder.
 17. The burner is set below 8 on the vertical scale.
 18. A nitrous oxide-acetylene flame is being used.
-

Set up conditions that relate to the diagnostic rules:

19. A refractory element is present in the solution.
 20. The samples are viscous.
 21. The uptake rate is more than 5 mL/min.
 22. Fluctuating display of lamp intensity while optimizing lamp.
 23. The hollow cathode lamp is on but there is no display of lamp intensity while optimizing lamp.
-
-

	Contamination from samples	Improperly positioned glass bead	Burner rotated by 90 degrees	Scale expansion set too high	Contaminated spray chamber	Precipitation in the nebulizer	Incomplete mixing of sample & fuel	Too few atoms in the flame	Background absorption too high	Liquid build up-spray chamber	Burner slot blocked
DATA QUALITY CONTROL											
Relative standard dev high	T	T			T	T	T	T	T	T	T
Noisy absorption signal		T		T				T	T	T	T
Abs values 50-80%		T			T	T	T		T	T	
Drift in baseline										T	
Change in absorption sensitivity	T		T	T	T	T	T	T	T	T	T
Calbn graph levels off					T	T		T			
Abs values 20-40%			T					T			T
Calibration graph sigmoidal											
Flame - ragged appearance					T	T				T	T
Pulsating signal							T			T	
Bubbles form in capillary tube											
Occasional pulse	T				T						
Decaying signal						T					
INSTRUMENTAL CONDITIONS											
Pressure <70 psi											
Oxidizing flame	?	?		?	?	?	?	?		?	?
Integration time too small		?	?	?	?				?		?
N ₂ O-acetylene flame	?	?	?	?	?	?	?			?	?
Burner > 8 on vertical scale											
No display - HCL on											
Fluctuating display- lamp intensity											
CHEMICAL CONDITIONS											
Sample is viscous										?	
Uptake rate less than 5 mL/min	?					T		T			
Refractory element present											

Table 2. The knowledge matrix in AAdiagnosis.

GP8 **THE PREPARATION OF STANDARD REFERENCE AQUEOUS SOLUTIONS (SRM) FOR HIGHLY HYDROPHOBIC MATERIALS.** Barry G.Oliver*, ZENON Environmental Laboratories Inc., 8577 Commerce Court, Burnaby, BC V5A 4N5 and M.Glenys Foster, ZENON Environmental Laboratories Inc., 5555 North Service Road, Burlington, Ontario L7L 5H7

1.0 Introduction

This study involved the development of standard reference aqueous solutions of highly hydrophobic materials using generator columns. Pioneering work in the area was done at the National Bureau of Standards in Washington by May and several co-workers. As a result of their early work generator columns for several PAHs are available from NBS. The main application of the columns has been to estimate aqueous solubilities and octanol-water partition coefficients of organic chemicals.

The analysis of trace organic in water and effluents is a research area of growing interest. Most analytical techniques are proofed by spiking samples in the laboratory to check recoveries of the various extraction and cleanup steps required to isolate the chemicals of interest from the matrix and from interferences. The weakness of this approach is the lack of knowledge of the stability of the analyte in the sample during the collection, transportation and storage. Generator columns can be used to produce aqueous solutions of hydrophobic organics at known concentrations so that the entire analytical scheme can be tested. These accurate solutions can also be used in interlaboratory studies to test performance of various laboratories.

2.0 Generator Column Preparation

Chromosorb W (60/80 mesh) was used as the column support. The Chromosorb W was first cleaned by placing 2 g of resin in a column and then passing 100 mL of methylene chloride and then 100 mL of hexane through the column at a flow rate of about 10mL/min. The chemical of interest was dissolved in hexane. Two grams of Chromosorb W was then placed in a flask with 100 ml of hexane containing 20 mg of the study compound. The hexane was then removed by rotary evaporation. The support place is thus coated with 1 percent by weight of the study chemical.

The coated Chromosorb W is then packed into a stainless steel column of dimensions, 0.64 cm outside diameter by 25 cm long, using a vibrator to assure tight packing. Both ends were sealed using stainless steel HPLC filter frits and Swagelok fittings. Each column contained about 1 gram of Chromosorb W and 10 mg of the study chemical.

2.1 Column Testing Procedures

Columns were attached to an HPLC pump with a delivery capacity of up to 15 mL/min. The column was suspended in a water bath which controlled the temperature to ± 0.1 °C. A variety of flow rates and temperatures were used for column #1 containing hexachlorobenzene (HCB) to study the effect of these variables. Subsequently, an optimum temperature and flow rate were chosen for testing of additional columns. High purity lab water (filtered/carbon absorption/reverse osmosis/ion exchange) was passed through the columns. Volumetric flasks (50 mL or 500 mL) were used to collect the solutions from the column.

2.2 Analytical Procedures

All standards for this study were prepared from high purity crystals obtained from US EPA at Triangle Park, North Carolina. At least 20 mg of crystals were weighed out on a Mettler Model HK60 balance that could be read to ± 0.01 mg.

Hexachlorobenzene, aldrin and endosulfan the 50 mL aqueous solutions from the columns were then placed in a separatory funnel and extracted with 25 mL hexane. The very low water/solvent ratio assured that greater than 99% of the chemicals were recovered. The hexane layer was dried with anhydrous sodium sulphate and placed in autosampler vials. Four vials from each sample extract were analyzed and the results averaged to obtain each data point. No cleanup or concentration of the extract was performed so no analyte losses would be expected. The analyses were performed using a Hewlett Packard Model 5880A gas chromatograph equipped with an electron capture detector.

3.0 Results

The experimental results from the four study compounds hexachlorobenzene(HCB), aldrin, endosulfan 1 and mirex are compared to literature solubilities in Table 1. In general, agreement between the current generator column produced aqueous solubilities with the available literature solubilities is good.

Table 1. A Comparison of Water Solubilities From This Study to Literature Values* (Concentrations in ug/L).

<u>Chemical</u>	<u>Solubility</u> <u>This Study</u> <u>(20°C)</u>	<u>Literature</u> <u>Solubility</u>	<u>Method</u>	<u>Source</u>
HCB	6.1±0.27	20(?°C) 6(25°C) 47(25°C) 5(25°)	SF** SF** GC*** SF**	Laseter et al(1976) Metcalf et al(1973) Miller et al (1984) Yalkowski et al(1979)
Aldrin	23.5±2.8	27(27°C) 17(25°C)	SF** SF**	Park and Bruce(1968) Weil et al(1974)
Endosul. I	470±13	164(?°C) 150(22°C,pH7.2) 260(20°C,pH5.5) 530(25°C) 600(?°C)	SF** SF** SF** SF** SF**	Ali(1978) Phillips(1975) Phillips(1975) Weil et al(1974) MRI(1977)
Mirex	0.048±0.004	1(?°C,0%Salin.) 0.04(?°C,4%Sal.) 0.007(?°C)	SF** SF** SF**	Alley(1973) Alley(1973) Smith et al(1980)

**SF = Shaker Flask Method.

***GC = Generator Column Method.

4.0 Summary and Conclusions

1. Methods for preparing generator columns have been detailed and this technology will be transferred to MOE in a report and through telephone conversations.
2. The aqueous solubilities of HCB, aldrin and endosulfan I have been measured and with the generator columns at 20°C.
3. The effects of flow rate and temperature on these solubility of HCB have been determined.
4. Some preliminary experiments were conducted with mirex and minor difficulties in using the procedure for chemicals with extremely low solubilities were found. These difficulties should be worked out using mirex before moving to the chlorinated dioxin part of the project.
5. Some successful testing was carried out using column with a lower chemical loading 0.05% instead of the usual 1%. This work indicates it may be possible to use much lower amounts of toxic chemicals such as chlorinated dioxins and still produce the desired saturated aqueous solutions.
6. Three generator columns have been delivered to MOE to deliver the following:

Column #1 Hexachlorobenzene(20°C) 6.1 ± 0.27 ug/L
Safe Water Volume = 800 liters

Column #2 Hexachlorobenzene (20°C) 6.1 ± 0.27 ug/L
Aldrin (20°C) 23.5 ± 2.8 ug/L
Safe Water Volume = 200 liters

Column #3 Endosulfan I 470 ± 13 ug/L
Safe Water Volume = 10 liters

DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR THE RAPID DETECTION AND QUANTIFICATION OF GLYPHOSATE

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INTRODUCTION

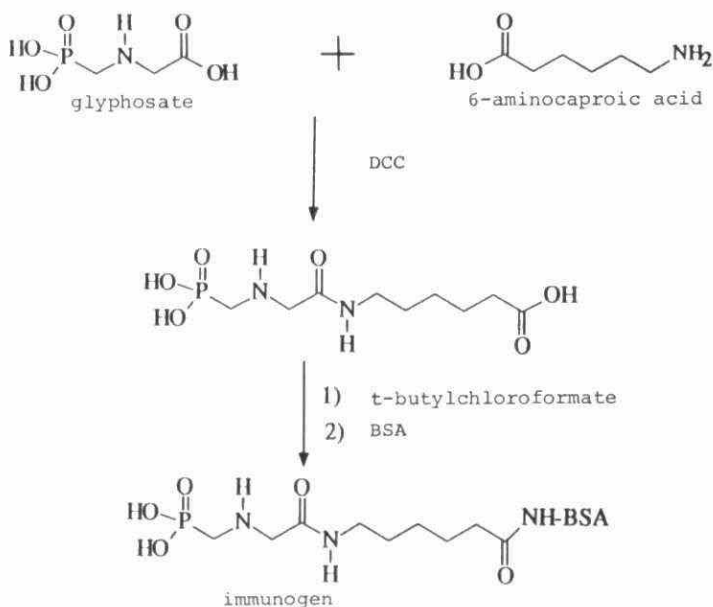
Glyphosate (N-phosphonomethylglycine) is a broad spectrum, non-selective, post-emergence herbicide; it is effective in the control of a wide range of weeds and at the same time is relatively non-toxic to mammals. The LD₅₀ based on oral feeding of male rats was reported to be 4320 mg/kg (Watts, 1980). The currently accepted LD₅₀ value for mammals is 1568 mg/kg (Miles et al., 1986). Properties which make this compound effective include high water solubility, rapid absorption and translocation by plants, and low degrees of *in vivo* metabolism and degradation. It is a unique compound in that it acts by disruption of phenolic metabolism (Hoagland and Duke, 1981).

Because of the significance of glyphosate, sensitive, accurate and widely applicable analytical methods for glyphosate are essential. Special complexities arise in the quantitation of this compound because it is soluble in water and insoluble in organic solvents. Currently, the detection of glyphosate is most commonly performed by biphasic aqueous-organic extraction followed by cleanup of the supernatant aqueous extract utilizing, first, iron-loaded Chelex 100 resin, and then, AG-1-X8 anion-exchange resin columns. Liquid chromatography coupled to a postcolumn reactor specific for primary amines, or compounds that can be converted to primary amines, is then used for the quantitation of the extract (Cowell et al., 1986). This analytical technique is long, costly, and has a detection limit near 0.05 ppm and recoveries around 80%. The use of an enzyme immunoassay would be of great help in i) shortening the time required for the analysis, ii) allowing the simultaneous analysis of many samples at the same time, and iii) lowering the detection limit. This research project is directed to the development of an enzyme immunoassay against glyphosate using polyclonal antibodies that could possibly, later be replaced by monoclonal antibodies. It is divided into three major stages which include: i) the preparation of the immunogen, ii) the production and selection of polyclonal antibodies and, iii) the preparation of a tracer and the development of a competitive enzyme immunoassay. Currently, part i) and some of part ii) have been achieved.

MATERIALS, METHODS AND RESULTS

1. SYNTHESIS OF THE BOVINE SERUM ALBUMIN-GLYPHOSATE CONJUGATE

As glyphosate is too small a molecule to be immunogenic, the first step in the production of an immunoassay for glyphosate is the synthesis of an immunogen consisting of the hapten, glyphosate, covalently linked to a large carrier molecule such as bovine serum albumin (BSA). The antibodies raised against such an immunogen tend to have highest specificity against that part of the hapten molecule that is furthest removed from the carrier. This consideration caused us to link glyphosate via the carboxylic acid function of the glycine moiety which will allow as much as possible of the phosphonate group to be exposed during antibody production. Molecular modelling calculations that predict the degree of steric hindrance of the hapten and the optimum electronic distributions for antibody formation indicated that the hapten should be attached to BSA via a five to six carbon chain rather than directly.



This was accomplished using 6-aminocaproic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) to form an amide. The amide was purified by preparative thin layer chromatography and methanol recrystallization and its identity confirmed by nuclear magnetic resonance and mass spectrometry. The glyphosate-caproic conjugate was attached to BSA by a mixed anhydride method using t-butylchloroformate. The resulting immunogenic conjugate was purified by dialysis against distilled water and subsequent lyophilization.

2. POLYCLONAL ANTIBODY PRODUCTION AND SELECTION

A set of six rabbits was used for the immunization. Blood samples were taken through the ear at DAY 0 in order to obtain pre-immune serum from each of the rabbits. They were then injected with 0.5 mL of a mixture 1:1 of the conjugate in PBS and complete Freund adjuvant (approx. 1 mg/mL). Four (4) injections of 0.5 mL each were made on each animal at the same time, two injections sub-cutaneously and two injections intra-muscularly. On DAY 21, 42, and 63, the rabbits were re-injected with the same amount of conjugate, but this time, mixed 1:1 with incomplete Freund adjuvant. On DAY 73, each rabbit was sampled through the ear and tested for the presence of anti-BSA antibodies using simple immunodiffusion. Since no reaction was observed, the six rabbits were re-injected twice following the same protocol in an attempt to raise the titer. They were then re-tested by immunodiffusion against BSA and also the BSA-glyphosate conjugate. Again, there was no reaction observed between the sera and the BSA alone. However, for two rabbits, precipitation bands were observed between the sera (rabbits number 3 and 6) and the BSA-glyphosate conjugate. These two rabbits were then bled by intra-cardiac puncture. Their sera is now being tested for specificity against glyphosate using a radioimmunoassay (RIA).

The RIA testing of the rabbit sera is as follow. Polyvinyl chloride microtitration plates are coated with 100 μ L of a 1,000-fold dilution in PBS (20 mM) of swine anti-rabbit immunoglobulins (Dakopatts, Denmark) and incubated overnight at room temperature (RT). Uncoated plastic is then saturated by adding 100 μ L of PBS-BSA (6%) to the wells. After an incubation of 3 h at RT, the plates are washed three times with PBS and 100 μ L of serial dilutions, in PBS, of the rabbit antisera are placed in wells. The plates are left at RT for 3 h. They are then washed four times with PBS-Tween 20 (0.05%) (PBST) and filled with 100 μ L of 14 C radiolabelled glyphosate (approx. 50,000 cpm) in PBST. After 3 h of incubation at RT, the plates are washed six times with PBST and each well is cut and placed in a liquid scintillation vial containing 12 mL of Beckman's Ready Protein⁺ cocktail. Radioactivity present in each well is then estimated using a Packard 1900CA Liquid Scintillation Analyzer. Antibody titer is

defined as the reciprocal of the amount of antisera (mL) required to give 50% binding of radioactive glyphosate.

Results were not yet available from the RIA testing when the Proceedings manuscripts were required.

DISCUSSION

The power of immunochemical technology to solve problems in the pesticide field is becoming widely appreciated. Immunoassays are physical assays which offer many advantages, including simplicity, sensitivity and specificity. Immunoassays do not rely on volatility (GC), thermal stability, or the presence of chromophores (UV, fluorescence) or heteroatoms (ECD, NPD) for detection. The development of competitive enzyme immunoassays is one of the most promising approaches to the detection and characterization of both xenobiotics and biological entities. Not only does enzyme immunoassay development allow the lowering of detection limits for environmental pollutants, but it also allows spending less time doing the routine runs and permits its application in less fully equipped laboratories. Since immunoassays usually work well in human body fluids, the technology is applicable to evaluation of worker exposure as well. Thus, immunochemistry is likely to complement existing technologies in providing, at reduced cost, the type of data currently generated in pesticide trace analysis.

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EXPERT SYSTEM FOR USE IN ENVIRONMENTAL CHEMISTRY. DESIGN AND IMPLEMENTATION OF GC-MSexpert AND AAexpert

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ABSTRACT

Expert systems will play an essential role in the environmental laboratory in the future. In this paper, we describe progress towards the development of two major expert system modules (i) AAexpert, a program that can control an atomic absorption spectrometer and associated solution handling instrumentation, can analyze the quality of the analytical data measured and can provide advice on corrective action to be taken to improve data quality, and (ii) GC-MSexpert, an expert system, which is designed to assist in the interpretation of GC-MS data associated with environmental samples. This system includes structure elucidation, identification of components and GC-MS data processing. Both these programs form part of ACexpert, a rule-based expert system with a graphic user interface written within Microsoft Windows 3.0. ACexpert incorporates several expert systems, including ACselect, AAexpert and GC-MSexpert. This paper describes the design of the prototype for these expert systems and a novel spectral data handling module.

Introduction

Expert system are ideal as tools to help solve complicated practical problems that require considerable prior technical knowledge. Expert systems are able to apply reasoning and problem-solving techniques to solve real-world problems. Applications within analytical chemistry fall into two broad categories: (i) systems that are developed to interpret experimental results, and (ii) systems that provide guidance or advice as to the course of action a analyst should take [1-4]. Specific programs include analysis of IR spectra [5], ^{13}C NMR [6], MS/MS [7], GC/IR/MS [8], methods development in HPLC [9,10] and automated metal analysis [11-14]. The promise in the development of expert systems for use in analytical and environmental chemistry is that the rules and facts in the knowledge base of an expert system can be assessed by experts in both regulatory agencies and industry, and the advice given to a wide body of user can be verified. An advisory role for expert systems appears at present to offer the greatest productivity gains. Typical roles for expert systems involve diagnosing instrument faults, diagnosing problems in instrumental measurements, advising on method development in chromatography, advising on complex data analysis and analyzing databases for hidden trends. These latter examples are of direct interest when environmental monitoring programs require large numbers of measurements to be made at frequent intervals: the quantity of data can overwhelm resources available to the analyst.

One of the most important aspects of an expert system is the implementation of the user interface. In an advisory role communication between the system and the user must be extremely flexible so that the flow of information is most efficient. We find that the breadth of information needed by the program in order to offer advice when systems are developed for use in the analytical laboratory, requires a very powerful user interface in which the user can monitor and adjust the information currently held by the system in response to the advice offered.

Results and Discussion

ACexpert This system incorporates a number of interrelated modules, of which ACselect can be used to determine the best method to use in the analysis of unknown compounds, and AAexpert and GC-MSexpert are programs that incorporate complete advisory systems. Figure 1 shows the structure of ACexpert.

ACselect ACselect is an expert system that aids in the selection of the optimum analytical technique to be used in the analysis of complex samples. ACselect has been developed by combining the ACexpert user interface with an "analytical techniques knowledge base" through an inference engine provided by the KDS shell. ACselect is subdivided into different sections, for example, AAmethods, which provides detailed information about AAS methods, and GC-MSmethods which provides similar information for analysis by GC-MS techniques. The object of this separation of method selection is that within an analytical laboratory there may be several techniques capable of making the determination. A number of parameters must be considered when the choice is made, amongst these are the required detection limits, freedom from interference due to the supporting matrix, cost per analysis, and regulatory agency requirements.

Selecting a proper analytical method is a more complicated procedure than simply accessing a set of information from the knowledge base. ACselect itself is an expert system, which not only consists of a knowledge base comprising the analytical information, but also a set of rules

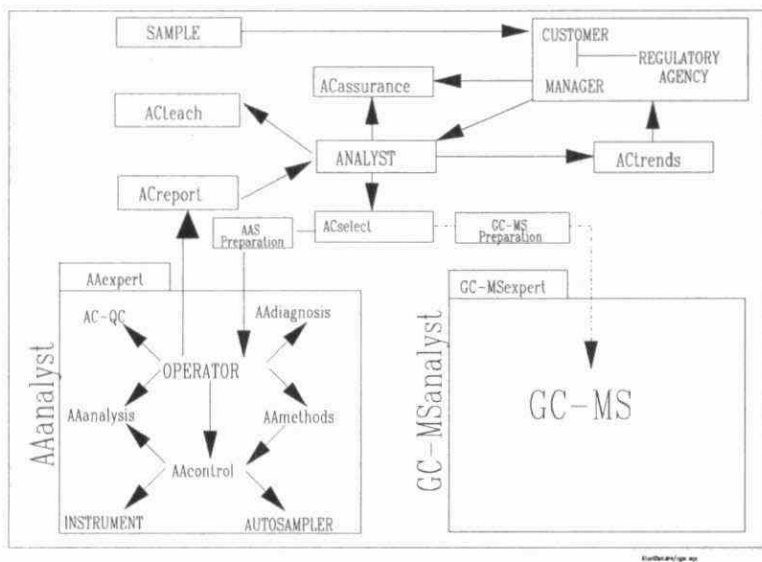


Fig. 1. ACexpert

that link these data together. The inference engine matches the users requirements for the sample, such as species, matrix, concentration range etc., with rules in the knowledge base in order to offer advice about the best analytical method. The value of expert system modules like ACselect, is that future regulations concerning environmental protection can be issued as a knowledge base on disk to be used by the managers of analytical laboratories to design monitoring protocols.

AAexpert Trace metal concentration determination using AAS (atomic absorption spectrometry) requires the completion of several tasks, each of which require extensive knowledge and expertise. The demands of regulatory agencies in the area of environmental protection, in particular, are such that precise selection of methods and the implementation of rigorous quality control is essential. Expert systems are ideal under these conditions as the evolutionary changes in methods and regulations can be added to the knowledge base without rewriting the program. Matrix effects, physical interferences, and spectral interferences are among the analyst's expert knowledge that the program must be able to assess as it decides on the advice to offer the user. Although incorporation of an expert system into the analytical laboratory will change how some of these tasks are carried out, the judgement and expertise of an analytical chemist or a skilled technician are still be needed to interpret the information offered by the computer system. The functions of the individual expert systems within AAexpert have been outlined previously [11-14].

EAexpert EAexpert is a complex expert system that controls the results obtained from a series of expert systems that carry out data analysis of data obtained by a number of chromatographic techniques. Figure 2 shows the structure of this expert system. Individual modules carry out data analysis using both spectral libraries and external databases to provide the best suggestion for the identity of the compounds represented by the spectral data. EAmaster then combines the results of each module and determines if a unique compound or class of compound can be identified. In this figure, we show the mix of procedural routines (for example, data conversion from instrumental formats to a common database format and data reduction) and rule-based routines. We briefly describe here the design of the system and features of the GC-MS data analysis module, GC-MSexpert, which is designed to give advice during GC-MS data analysis of measurements made on organic compounds in complicated environmentally-important samples.

Combined gas chromatography-mass spectrometry (GC-MS) is an established technique for the analysis of complex mixtures and because of its sensitivity, wide range of applicability and versatility, the GC-MS technique is widely applied in chemistry, medicine, biochemistry, pharmacology, environmental pollution control, and food science [15]. However, analysis of individual GC-MS data sets generally requires a specialized expertise. In order to deal more efficiently with this problem, GC-MSexpert has been designed as a rule-based expert system with a graphical user interface. There are three stages in our design:

1. Development of GC-MS data processing/data transfer facilities.
2. Implementation of a sophisticated graphical user interface.
3. Design of the knowledge base.

Analysis of GC-MS data can be very complicated and requires extensive knowledge and expertise. Moreover, a GC-MS analysis of samples from environmentally-important sources produces a tremendous amount of data, which requires an effective means of data management and analysis. The prototype described here is designed to resolve these problems. The GC-MSexpert system is designed to assist the analyst deal with the GC-MS analytical problems

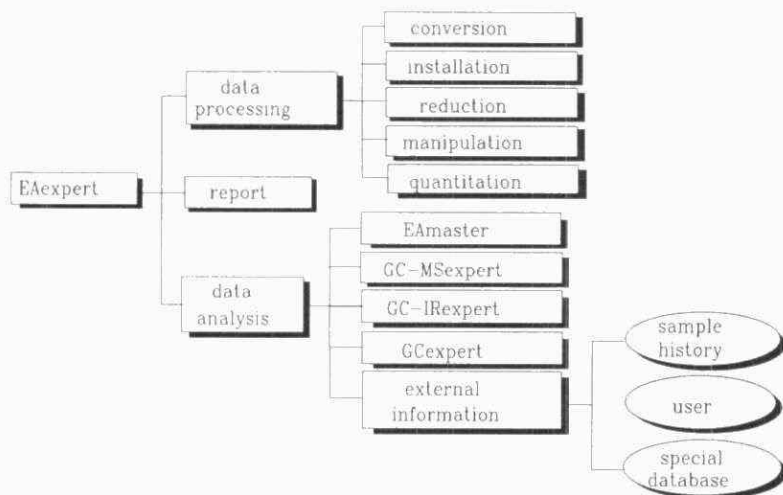


Figure 2. Outline of the EAexpert system

associated with the determination of compounds in the sample of environmental interest, carry out automatic structure elucidation and offer advice on the identification of components in the samples, and finally, provide a sophisticated tool for GC-MS data processing, which includes a spectral data archiving capability for data interchange between laboratories. An overview of the system architecture is shown in Figure 3. The program will act directly to carry out both rule-based and procedurally-based operations on GC traces and associated mass spectral data. The analyst initially can browse through the GC data with the system presenting selected mass spectral data in a graphical window. Baseline subtraction and alignment with data from standards can then be carried out. Band deconvolution, fragment identification and library searches, controlled by the rule-base, are used to identify individual components in the chromatography of the analyte. Usually, if the mass spectrum of a substance of unknown structure has been obtained, the most popular computerized method of determining the structure of the unidentified compound is to search through a library of known spectra for a match between the sample spectrum and that of a compound in the library. When spectral data from the unknown compound are not present in the library, library searching techniques are of limited use. One approach to solve this problem, is to propose a structure that is consistent with the given spectrum. However, it may be possible to propose several structures that are compatible with the spectrum. The proposed structures must be verified by a variety of other methods.

Implementation of the user interface Expert systems generally include a knowledge base, an inference engine and a user interface. The user interface handles all communication between the user and the different modules in the expert system. An appropriate and user-friendly interface is vital for the success of an expert system that is to be used as an advisor. Since the analysis of GC-MS data usually involves complicated data interpretation and significant

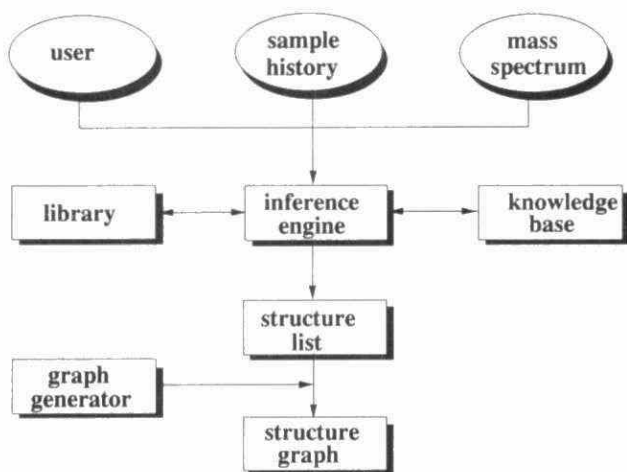


Figure 3. Structure of GC-MSexpert

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mathematical processing, the requirements for the user interface include a component to aid in managing the complexity of these tasks. The GC-MSexpert system is implemented within Microsoft Windows™ 3.0, which provides a multitasking, graphical-based windowing environment.

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DESIGN AND IMPLEMENTATION OF ACselect

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ABSTRACT *This paper describes the application of expert system technology to analytical chemistry as part of the development of the ACexpert system. The success or failure of an analysis often critically depends upon the proper selection of a method, ACselect is an expert system that advises the analyst how to solve real-world problems based on sample analysis information. A prototype of ACselect has been developed in our laboratory which encodes the technique for choosing a proper technique for a required analysis. ACselect has been developed by combining the ACexpert user interface with an Instrumental Analysis database through the inference engine provided by the AI shell, KDS. ACselect is a rule-based expert system written within ACexpert system that uses a Windows 3.0™ interface.*

Introduction

The standard approach to solve an analytical problem is largely heuristic, with past experience and rule of thumb being used. Such rules do not have general applicability and only experience can tell whether or not to apply one in a given situation. Expert system can combine theory with rules to provides the flexibility necessary to cope with complex problems of the real world¹. These systems will not replace chemists, but rather will assist them in performing their daily work².

The development of an expert system must start with some concept of what an "expert" is, how experts perform scientific tasks, and how scientific "expertise" is used by the expert. The unforeseen obstacle in developing expert systems is the difficulty in extracting knowledge from human experts and in representing knowledge in a logical and consistent format. This problem is often referred to as *the knowledge acquisition bottleneck*¹⁵. The value of our present research is to find a way to extract expertise from human experts and reformat into a rule-based knowledge structure which the computer can use.

Structure of ACexpert

Expert system applications within the field of Analytical Chemistry³⁻¹³ fall into two broad categories: (i) Systems that are developed to interpret experimental results, and (ii) systems that provides guidance as to the course of action the analyst should take.

ACexpert, which is a rule-based expert system with a graphical user interface, has been designed to provide real-time assistance to the analyst in completing a required analysis. ACexpert incorporates several individual expert system modules, including ACselect, AAexpert, and GC-MSexpert (Figure 1). Each of these is capable of acting independently or as a module of the full system.

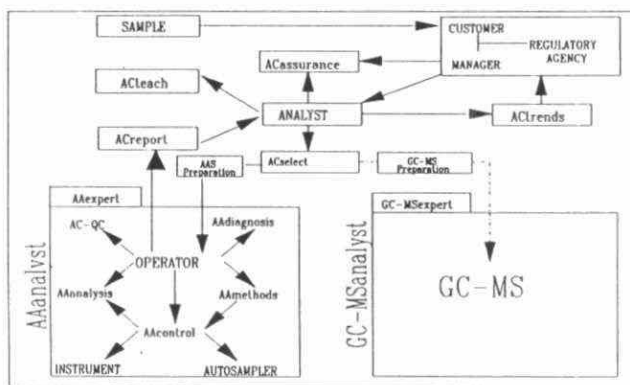


Figure 1. The Structure of ACExpert
ACselect

The general stages in an analytical process are: (1) Sampling; (2) Separation and preparation; (3) Selection of an analytical method; (4) Performing the analysis; (5) Quality control/Quality assurance; and (6) Reporting the results. Clearly, this list does not indicate any of the complex interactions that exist between the various steps. Although each of the above steps is almost always involved in an analysis, there is no definitive algorithmic approach that can be applied in every situation.

The expert system shell KDS-3 (KDS Corporation) has been chosen within which our expert system will be developed. As an inductive shell, KDS works in the sequence of facts...rules...knowledge, the "step by step" example-driven programming structure makes KDS an excellent environment for resolving the ambiguity of unclear rationalizations¹⁴.

Selecting a proper analytical technique is more involved than simply accessing a set of information from the database. ACselect itself is defined as an expert system, which not only consists of the knowledge base where all the analytical chemistry information is stored in the form of rules and facts, but also has a built-in inference engine provided by the KDS shell, which matches the requirements of the user through the ACExpert user interface and the proper information selected by the AI shell from the database.

Whenever confronting an analytical problem, an analyst accumulates a set of information before he/she can make any further decisions. This information includes: What is the sample type, such as: inorganic or organic, pure or mixture, elemental or

molecular? What is the matrix, or sample media? What is the concentration range? No matter what method is chosen, all these questions must be answered before a correct conclusion can be reached. If $F(x,y,z)$ can be used to represent an analytical case, then we call the unknown factors (x , y , and z) the "Degree of Freedom (DOF)" for this particular analytical problem. Accordingly, ACselect is also broken down into sub-modules, each of which performs a single task, which aims to eliminate one of the DOFs in each analytical problem. A general module holds all these pieces together to form a complete program (Figure 2).

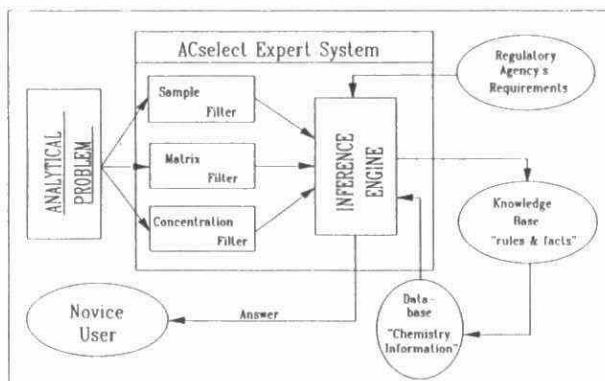


Figure 2. The Structure of ACselect

It is clear that through a series filters, the DOFs become eliminated, and from this point, combined with the requirements of the regulatory agency or the customer, a clear and detailed profile about the sample begins to emerge. This computerized information can then be used by the computer as a direction that the inference engine uses with the rules and facts from the knowledge base to rationalize the generation of the proper method(s) for the novice user.

Discussion

We have completed AAdiagnosis using KDS (see the paper by Lahiri and Stillman) and have developed new techniques with which to code the heuristic knowledge required by ACselect. Although ACselect is mostly still in the design stage, we did try several knowledge modules based on the ideas explained previously. Because the computer program can not "think", the focus really is how intelligent can the program be? ACselect cannot accomplish its task by simply accessing a database. The choice of a technique and subsequently a method is too complicated for that approach. Our study aims at combining rules and database access in one program in order to provide that program the ability to offer expert advise.

Acknowledgement

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CHARACTERIZATION OF THE EXTENDED CAPABILITIES OF ICP-MS WITH FLOW INJECTION INTO A GASEOUS CARRIER. Diane Beauchemin and Yves Le Blanc, Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Although one of the features of inductively coupled plasma mass spectrometry (ICP-MS) is its low detection limits for a large number of elements, the quantitative determination of As, Se and Hg at 0.2 $\mu\text{g/L}$ in ground water is a task which is still impossible, on a routine basis. This situation results from several factors. First, all those elements possess a degree of ionization $\leq 50\%$ in the argon plasma (1). Secondly, the plasma produces background peaks directly overlapping three of the six isotopes of Se (at m/z 76, 78 and especially 80), which further hampers its determination since all the remaining isotopes of Se have a relatively low natural abundance. Isobaric interferences from argon-containing polyatomic species may also degrade the detection limit for As if any chlorine-containing compound is part of the matrix since it would lead to the formation of $^{40}\text{Ar}^{35}\text{Cl}^+$ which directly overlaps the only isotope of As. Finally, Hg possesses six isotopes which all have a natural abundance lower than 30%. The determination of this element is also plagued by a large and persistent memory effect (2) which unduly raises the background unless excessively long wash-out times are used between samples.

In order to enable the simultaneous quantitative determination of these three elements with a determination limit (i.e. 5 to 10 times the detection limit) of 0.2 $\mu\text{g/L}$, changes in the operating conditions of the instrument are required. Ideally, these changes should result in: 1) an increase in the degree of ionization; 2) a reduction of isobaric interferences from polyatomic species containing Ar; and 3) a reduction in memory effects. Several approaches have been used towards each of these goals but, so far, none has aimed at the three of them simultaneously.

Mixed gas plasmas have been shown to enhance the ionization process (e.g. 3-4). For instance, Lam and Horlick (3) reported that the addition of N_2 , air or O_2 to the plasma gas in ICP-MS improved sensitivity at smaller sampling depth, if the nebulizer flow rate and the power were increased. Although in their work and that of others, the other gas was mixed with Ar prior to introduction into the torch, other approaches can also be used. For instance, Murillo and Mermet (4) reported that an addition of H_2 as sheath around the nebulizer gas flow enhanced the ionization process. No matter how the gas is added, the enhancement observed with mixed gas plasmas results from the higher thermal conductivity of molecular gases which provides a more efficient transfer of energy within the plasma (4).

Mixed gas plasmas can also lead to a substantial decrease in isobaric interferences from Ar-containing polyatomic species. For example, Evans and Ebdon (5) as well as Branch et al. (6) reduced drastically the interference from $^{40}\text{Ar}^{35}\text{Cl}^+$ on $^{75}\text{As}^+$ by adding a small amount (30 mL/min) of N_2 to the nebulizer gas flow rate.

Desolvation can be used to further enhance the degree of ionization and reduce interferences from polyatomic species. For instance, Lam and McLaren (7) reported a substantial reduction in $^{40}\text{Ar}^{16}\text{O}^+$ (which overlaps directly with the major isotope of Fe) by adding N_2 to the plasma gas and desolvating the aerosol prior to its introduction into the plasma. Not only does N_2 scavenge oxygen (6), but the reduced solvent load decreases the amount of energy required for the desolvation process, leaving more energy available for the ionization and/or excitation processes.

As far as memory effect is concerned, the most straightforward way of reducing it is to decrease the amount of sample being introduced into the plasma. Flow injection (FI) is a very convenient and simple way to achieve this (8) since it consists in injecting discrete amounts of sample (for example, 100 μL) in a flow of carrier (typically water). Obviously, aspirating a few microliters of a solution containing Hg cannot lead to as large a memory effect as continuously aspirating the same Hg solution. Furthermore, the carrier which immediately follows each sample plug has a continuous rinsing effect. FI can be used to minimize the memory effect of Hg (2). Moreover, if the distance between the sample injection valve and the nebulizer is kept small, only a limited dispersion of the sample occurs as it moves towards the nebulizer, resulting in little loss of sensitivity (i.e. a factor of 2 at the most, for 100- μL injections (8)) compared to that observed by continuous aspiration. FI also has numerous additional advantages such as: increases sample throughput, drastic reduction of clogging of the interface by deposition of solids on the sampler and skimmer, minimal sample consumption, etc. (8).

In principle, combining the advantages of FI with those of mixed gas plasmas, should fulfil the three requirements. Ideally, desolvation should also be included to truly maximize the benefits from mixed gas plasmas. A straight combination of the equipment required for FI, mixed gas and desolvation can be made, but it results in a somewhat expensive and elaborate set-up including a peristaltic pump, sample injection valve, some tubing and connectors for FI; a gas proportioner (or a sheathing device if the sheathing gas approach is used) for mixing gases and a heating chamber/condenser system for desolvating the aerosol. A much simpler alternative way is to use a gas as the carrier in FI. Besides from being less expensive, this approach has several other potential advantages. In this way, a gas (such as air or N_2) can be introduced directly into the centre of the plasma, which should reduce the amount of Ar sampled from that region and result in a lower background (i.e. reduced interferences due to Ar-containing polyatomic species). Since the gas is used as the carrier, no dispersion of the sample occurs on its way to the nebulizer, so the sensitivity should be at least the same as that with continuous aspiration. Finally, since only discrete aqueous sample plugs reach the plasma, the solvent load in the plasma will be reduced compared to continuously nebulizing aqueous solutions (but not as much as with desolvation).

During this presentation, FI into air as the carrier will be characterized. In particular, the effect of critical parameters such as the nebulizer flow rate, the uptake rate (i.e. the peristaltic pump flow rate) and the size of the sample loop will be considered. Advantages and disadvantages of the system will be assessed. The results will demonstrate that FI into a gaseous carrier not only substantially reduces the solvent load in the plasma, leaving more energy available for ionization of the analytes, but also enhances ionization. The precision (using peak height) is also improved compared to FI in water; and the sampling rate can be even greater than with FI in water.

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**AUTOMATION OF A DUAL OPEN COLUMN CHROMATOGRAPHIC CLEANUP TECHNIQUE
FOR SAMPLES CONTAINING CHLORINATED DIBENZO-P-DIOXINS AND
DIBENZOFURANS**

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A technique to automate the cleanup stage of samples analyzed for ultra trace levels (ppq) of chlorinated dibenz-p-dioxins (PCDDs) and dibenzofurans(PCDFs) has been researched by the Ontario Ministry of the Environment (MOE). The Fluid Management System (FMS - Nortech Controls) replaces a manual two stage open column cleanup technique used in the Dioxin Unit.

The FMS used in the Dioxin Unit at the MOE processes five samples unattended and automatically through a set of two columns. The first contains three treated silica gel packings and the second column contains alumina. Two fractions per sample are collected, the first contains polychlorinated biphenyls (PCBs) while the second fraction contains PCDDs and PCDFs.

This automated system saves the chromatographer a full day in the sample preparation process. When done manually, the dual column cleanup takes two day; one day per column set with a solvent concentration step in-between columns. The FMS will process one sample extract through two columns in one hour. Samples are run in series, so a set of five samples takes five hours. Once all columns are packed and sample extracts and glassware are in place the system is ready to perform the cleanup unattended.

This system has been optimized for dioxin and furan recoveries. These recoveries are equal to or greater than those achieved by manual column chromatography. No carryover has been observed provided column capacity has not been exceeded.

Method

Hand packed silica and alumina columns were placed in the positions shown in Figure 1. The first step in this automated cleanup was to wet the columns with hexane before sample addition. When this task was complete the sample was taken from a roundbottom flask and placed on the column. After sample addition, clean hexane was pumped from the solvent reservoir into the roundbottom before being eluted through the column. This step rinsed the roundbottom to help maximize surrogate recoveries.

Once all hexane rinses passed through the columns (silica and alumina) the dioxins and furans were present only on the alumina. Any PCB interferences present were removed with the 2% dichloromethane(DCM):hexane (v/v) elution of the alumina column. The PCDDs and PCDFs were then collected by elution with 25%

DCM:hexane (v/v). After collection was complete all of the common lines were flushed with hexane.

All of the steps described were carried out using the FMS which has a microprocessor to control the valve switching as well as the pumping system. Once a program had been written for each sample the valving control commands were saved and recalled at a later date.

Method development was carried out by spiking clean solvent with a $^{13}\text{C}_{12}$ -PCDD surrogate standard that had a 2,3,7,8 substituted isomer in each of the congener groups from tetra through to octa. The spiked solvent was then run through the columns as described above and the collected fractions were analyzed for recovery of the surrogate standard. Initial analyses were carried out by GC-ECD (Hewlett Packard 5890). Some of the carryover check samples were run by GC-MS (Finn 4500).

Experimental

An optimized manual method was used as a baseline for setup of the automated method. Recoveries of the PCDDs using the automated method were optimized using the silica columns alone (Table 1) followed by the complete silica/alumina system (Table 2).

Results and Discussion

TABLE 1 Percent Recovery of Spiking Solution on Silica Columns using FMS

Isomer	Volume of Hexane (mL)	
	130	160
$^{13}\text{C}_{12}\text{T4CDD}$	64	88
$^{13}\text{C}_{12}\text{P5CDD}$	56	73
$^{13}\text{C}_{12}\text{H6CDD}$	65	82
$^{13}\text{C}_{12}\text{H7CDD}$	67	101
$^{13}\text{C}_{12}\text{OCDD}$	63	75

TABLE 2 Percent Recovery of Spiking Solution on Dual Columns using FMS

Isomer	Volume of 2%DCM:hexane/ 25% DCM:hexane		
	20/50	20/50 ¹	20/50 ²
¹³ C ₁₂ T4CDD	87	103	49
¹³ C ₁₂ P5CDD	66	95	56
¹³ C ₁₂ H6CDD	70	107	44
¹³ C ₁₂ H7CDD	102	124	50
¹³ C ₁₂ OCDD	79	103	48

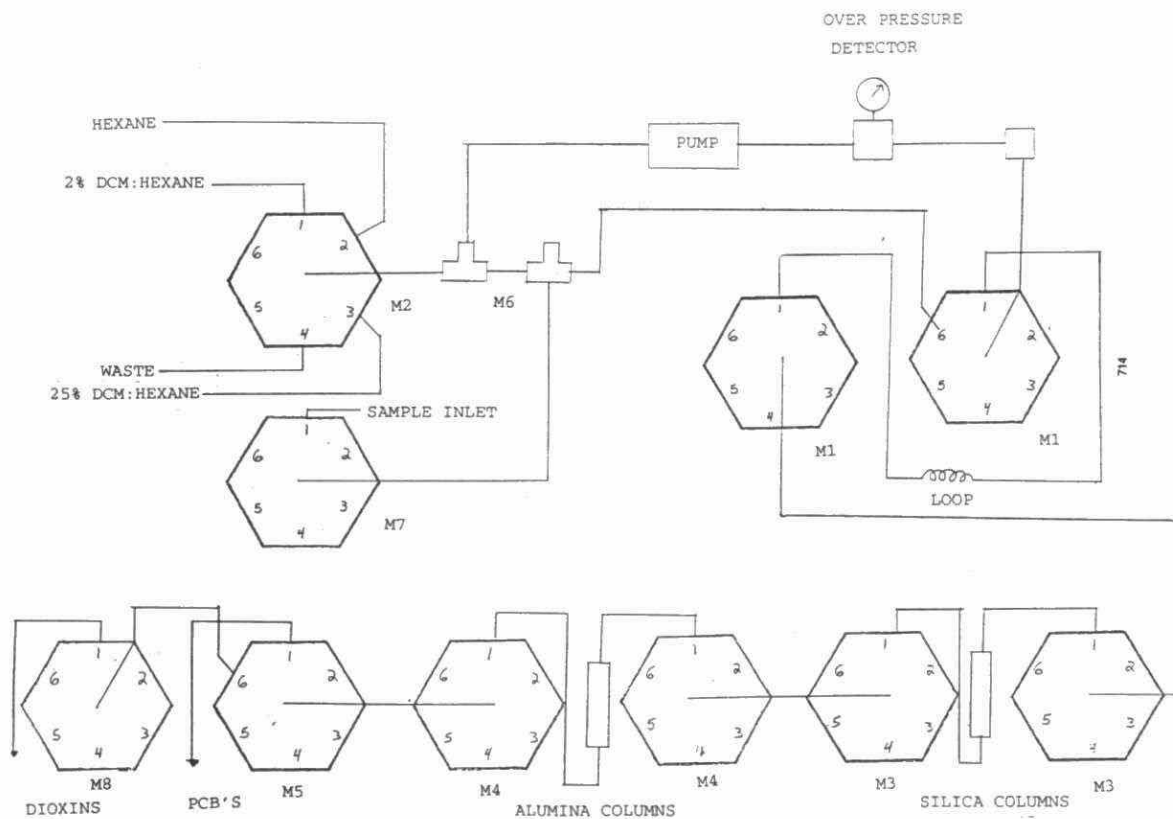
- 1 A five minute delay before elution of the alumina column was programed into the method to ensure all of the hexane eluted off the column before addition of the 2%DCM:hexane.
- 2 This column set was spiked with a flyash extract as well as the surrogate spike. The levels of dioxins and furans in the flyash were approximately 5 to 50 ng of tetra through octa.

Overall the recoveries are acceptable but should be improved to handle high level samples more efficiently. The high levels of dioxins and furans in the sample may have overloaded the columns.

A carryover study was done at the same time. The fraction collected from the column set following the flyash extract contained approximately 0.1 ng of hexa through octa dioxin. This works out to be a 0.2% carryover. If the volume of hexane used to rinse to common lines is increased the carryover may be eliminated.

Fine tuning of the method is still needed. Priliminary results look promising. The advantages to this system are significantly reduced preparation times as well as the reduced human exposure to highly toxic chemicals.

FIGURE 1. AUTOMATED CHROMATOGRAPHIC TECHNIQUE FOR ISOLATION OF CHLORINATED
DIBENZO-p-DIOXINS AND DIBENZOFURANS



AN AUTOMATED HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CLEANUP PROCEDURE FOR THE DETERMINATION OF CHLORINATED DIBENZO-P-DIOXINS AND CHLORINATED DIBENZOFURANS

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Introduction

The need for detection of ultra-trace levels of chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) has become a priority in environmental labs because of the possible human health concerns of the 2,3,7,8-chlorine substituted dioxin and furan congeners. CDDs and CDFs occur as byproducts in the manufacturing and combustion of various chlorinated compounds such as chlorinated phenols (eg. pentachlorophenol, which was used on a large scale in fungicides and wood preservatives), phenoxy herbicides, chlorinated diphenyl ethers, and polychlorinated biphenyls(1). The introduction of these pollutants into our waterways from industrial sources is evident when analyzing various biota samples.

CDDs and CDFs bioaccumulate readily in animal fat tissue because of their lipophilic nature and stable physical properties(2). Other chlorinated organic compounds are present in many samples at concentrations several orders of magnitude higher than those for CDDs and CDFs. To obtain acceptable detection levels, this particular analysis requires very sensitive and specific analytical techniques. After extraction of the analytes from the matrix, the bulk of the interferences are removed with an initial cleanup of the sample extract. An important class of interferences are chlorinated diphenyl ethers(CDPEs)(3)(4). These compounds fragment under electron ionization conditions in the mass spectrometer ion source to form ions isobaric with CDF molecular ions, producing false positive results. Low-resolution, high-resolution, or tandem mass spectrometers (LRMS, HRMS, MS-MS) cannot distinguish the CDFs from the CDPEs. Therefore the CDPEs must be removed prior to the GC/MS analysis. The Ontario Ministry of Environment (MOE) has developed a sample preparation procedure incorporating an automated dual column HPLC cleanup step to address this specific interference and this development allows the sample to be analyzed by LRMS techniques.

Experimental Section

Sample Preparation

Twenty grams of ground and homogenized fish tissue was weighed into an Erlenmeyer flask. Each sample was spiked in the flask with a surrogate standard solution containing five 2,3,7,8 substituted $^{13}\text{C}_{12}$ -labelled dioxin isomers, one from each congener group. The sample was then digested overnight (approximately 16 hours) in concentrated hydrochloric acid. The acid digested tissue was extracted with hexane and the extract was passed through a cylindrical funnel packed with anhydrous sodium sulphate and sulphuric acid impregnated silicic acid. The extract was concentrated and quantitatively transferred to a 100 μL conical vial and taken to dryness. The sample was then reconstituted in 150 μL of hexane and was ready for injection onto the HPLC system.

Instrumentation

The automated HPLC cleanup procedure involves the use of a column-switching step whereby the CDD and CDF fraction is trace-enriched from a neutral alumina HPLC column onto another HPLC column packed with a carbon-silica mixture. The CDDs and CDFs are retained on the carbon-silica column while interferences such as polychlorinated biphenyls, polychlorinated diphenyl ethers, and some polychlorinated naphthalenes are not retained. The CDDs and CDFs are recovered from the carbon-silica column by backflushing the column with toluene. The automated HPLC system consists of the following components, all of which are computer-controlled:

- Automated sample processor and injector
 - Gilson model 232-401
- HPLC pumps (3)
 - Gilson model 302 piston pumps
 - only 2 are used for forward elution
 - solvent used is hexane and dichloromethane
- Programmable HPLC pump
 - Gilson Model 305
 - solvent used is toluene
- Ultraviolet Detector
 - Gilson Model 116 - programmable
 - Fraction collector
 - Gilson Model 202
- Dynamic Mixer
 - Gilson Model 811B
- Manometric module
 - Gilson model 802C
- 6-port switching valves (2)
- HPLC columns (2)
 - Normal Phase Alumina, (0.46 cm ID x 25.0cm from Phenomenex, Torrance, CA, USA), Spherisorb with 5 micron particles

- Empty Waters guard column (0.46 cm x 3.0 cm) packed in-house with a mixture of 5%(w/w) Amoco PX-21 carbon/silicic 70-230 mesh)

After HPLC cleanup, the samples were concentrated and quantitatively transferred to 100uL conical vials. The samples were then ready to be analyzed by the following gas chromatography/mass spectrometry techniques.

Gas Chromatography Low Resolution Mass Spectrometry (GC/MS)

Fifteen fish samples were analyzed using a Finnigan 4500 GC/MS. Samples were introduced onto a 30m DB-5 fused silica capillary column(0.25mm I.D. with 0.25µm film thickness) via an on column injector at ambient temperature. The GC conditions were as described previously(5). The GC effluent was directly interfaced to the mass spectrometer. The instrument was tuned using perfluorotributylamine(PFTBA).

CDD's and CDF's were detected using selected ion monitoring (SIM) techniques. The ions monitored for the native CDDs/CDFs correspond to the three most abundant molecular ions. Only two ions are monitored for the $^{13}\text{C}_{12}$ -labelled surrogate standards. Two ions are also monitored with each congener group for the CDPEs.

Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS)

Twenty five fish samples were analyzed using a Finnigan TSQ70 GC/MS/MS. The samples were injected on a 60m DB-5 column (0.25mm I.D. with 0.25 µm film thickness), through a splitless injector, at 300°C. The GC was programmed as follows: hold initial column temperature at 120°C for 1 minute, ramped at 7.5°C/min. to 250°C, then ramped to 300°C at 2.5°C/min., and hold for 10 minutes. The carrier gas was helium, at a column head pressure of approximately 22 PSI. As previously described(6), the instrument was tuned using CDDs.

The interferences which affect LRMS sensitivity and selectivity are those compounds which are co-extracted with CDDs and CDFs and are not removed in the cleanup steps. MS/MS can be used to distinguish CDDs and CDFs from chemical interferences by selected reaction monitoring (SRM) techniques. MS/MS detection consists of the mass selection of a parent ion in the first mass analyzer, fragmentation of this ion by collision induced dissociation(CID) and final detection of the daughter ion in the second mass analyzer. Interferences such as CDPEs can not be distinguished from CDFs by MS/MS or any other type of mass spectrometry because under EI conditions they fragment to form ions isobaric with CDF molecular ions in the ion source.

Results and Discussion

The automated HPLC dual column cleanup method developed at MOE was found to be very effective in removing interferences such as PCBs and CDPEs. The recoveries of the internal standards were found to be acceptable and consistent, with a 62% average recovery. The extract was clean enough to allow the analysis to be carried out by LRMS techniques. The detection limits achieved by the Finnigan 4500(LRMS) were typically 4 to 5 PPT. Although these were good detection limits, a detection limit of 4 PPT is required to obtain "Toxic equivalent" detection limits below the levels required for a warning to be issued in the MOE/MNR "Guide to Eating Ontario Sports Fish". The sensitivity of MS/MS techniques was preferred because the detection limits achieved by LRMS were too close to the guideline. The detection limits obtained by MS/MS analysis were typically less than 3 PPT.

Further development will test the applicability of this cleanup procedure to other environmental matrices where CDPEs and other organic interferences are problems. Future work will also include the determination of the active lifetime of a carbon column and optimize the column for maximize lifetime.

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A STUDY OF CHEMICAL INTERFERENCES IN THE ANALYSIS OF N-NITROSODIMETHYLAMINE IN ENVIRONMENTAL SAMPLES.

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N-nitrosodimethylamine (NDMA) is also known as dimethylnitrosamine (DMNA) and N-methyl-N-nitrosomethanamine. NDMA can be present as a trace contaminant in industrial processes and in products manufactured using dimethylamine. It may also be found in various foods such as cured or smoked meats, beverages such as beer and in tobacco smoke condensates. In addition, NDMA has been found in some ion exchange resins. NDMA has been found to be carcinogenic in the mouse, rat, hamster, guinea-pig, rabbit and rainbow trout and is a suspected human carcinogen. Because of the suspected toxicity and carcinogenicity of NDMA, the interim drinking water guideline has been set by the Ontario Ministry of the Environment (MOE) at 9 ppt.

NDMA can be analyzed by a number of methods including gas chromatography (GC) followed by thermal energy analysis (TEA), low resolution mass spectrometry (LRMS) or high resolution mass spectrometry (HRMS).

The sensitivity of the instrumentation is sufficient to meet the drinking water guideline. However, the degree of selectivity required of the detection system may be predicated by the degree of sample cleanup utilized.

The sample preparation protocol developed at MOE was designed to be simple. Since NDMA is a neutral compound, the sample cleanup, after extraction from the matrix, is a partitioning scheme that removes the basic and acidic components from the extract. A summary of the protocol is outlined below:

d₆-NDMA is added to the sample as the internal standard. Filtration is used to remove any particulates that may be present. After the pH is adjusted to 12 to keep the acidic components in the aqueous phase, the basic solution is serially extracted with dichloromethane. The dichloromethane extract is washed with a sulphuric acid solution to remove basic components from the organic phase. The washed extract is filtered through granular anhydrous sodium sulphate to remove water and is then concentrated by rotary evaporator and a nitrogen evaporating unit.

The sample extract contains the remaining neutral organics and no further cleanup is done to separate neutrals from neutrals.

The sample extracts were analyzed by GC/LRMS and GC/HRMS.

The mass spectrum of NDMA consists of 2 major ions, the molecular ion at m/z 74 and an intense fragment ion at m/z 42. Selected ion monitoring (SIM) analysis of NDMA by GC/LRMS was limited to a quantitation ion at m/z 74 and a single qualifying ion at m/z 42. The acceptable ratio of m/z 42:74 was set at +/- 20% of the ratio obtained from a calibration standard run on the same instrument.

The mass resolution requirements for HRMS were determined as follows:

The exact masses for NDMA and a common ester fragment are m/z 74.0480 ($C_2H_6N_2O$) and m/z 74.0368 ($C_3H_6O_2$), respectively. The mass resolution required to differentiate these masses is 6,607 [$74/(74.0480-74.0368)$]. Operation of a high resolution mass spectrometer at 7,000 resolving power (RP) allows NDMA to be differentiated from chemical interferences such as $C_3H_6O_2$.

On a single quadrupole mass spectrometer, which is restricted to low resolution mass spectrometry (LRMS), ions having the empirical formulae $C_2H_6N_2O$ and $C_3H_6O_2$ are indistinguishable from each other because they both have $m/z = 74$. Therefore qualifying ion(s) must be used and the ratio of the qualifying ion to the quantitation ion must be within acceptable limits.

On a high resolution mass spectrometer, single ion monitoring of the accurate mass m/z 74.0480 provides sufficient selectivity to distinguish NDMA from chemical interferences.

Instrumental conditions were as follows:

	GC/LRMS	GC/HRMS
GC	HP5890	Varian 6000
MS	VG Trio-2 or HP 5970 MSD	VG ZAB-2F
Column	DB-1701 (30 m)	DB-1701 (30 m)
Electron energy	70 eV	70 eV
Ions monitored	m/z 42, 74 (NDMA) m/z 46, 80 (d_6 -NDMA)	m/z 74.0480 (NDMA) m/z 80.0857 (d_6 -NDMA) m/z 68.9952 (PFTBA)(lockmass)

Analyses of drinking water samples were undertaken. A comparison of analyses of drinking water extracts is shown in the following table:

LRMS vs HRMS Data ($\mu g/L$)

Sample	LRMS-A	LRMS-M	HRMS
1.	0.025	0.016	0.019
2.	0.023	0.015	0.012
3.	0.017	0.011	0.011
4.	-----	0.011	0.013
5.	-----	0.034	0.019

A = automatic areas

M = manual areas

These data demonstrate that:

- (1) automatic areas are dependent on the peak/baseline detection parameters chosen and the peak widths of overlapping interferences; peak areas may have to be determined manually (compare samples 1-3).
- (2) neutral chemical interferences in drinking water samples co-elute with NDMA.
- (3) confirmation by HRMS may be required.

Further studies included other matrices as shown in the following table:

LRMS vs HRMS Data (ug/L)

Sample	LRMS	HRMS
1. Spiked HPLC-grade water	0.49*	0.42
2. STP influent	0.43	0.40
3. STP influent	0.11*	0.02
4. STP influent	0.20*	0.24
5. Groundwater	ND	0.09
6. Groundwater	1600*	170
7. Industrial effluent	0.37*	0.26

* interference (incorrect ratio of m/z 42:74)

ND = not detected (no peak)

These data demonstrate that:

- (1) neutral chemical interferences in other matrices co-elute with NDMA.
- (2) confirmation or analysis by HRMS may be required.

Full scan GC/LRMS of a groundwater (sample 6) was undertaken to identify some of the neutral chemical interferences.

Chlorobenzene, ethylbenzene and xylenes were found to be present. Chlorobenzene and ethylbenzene co-elute with NDMA and d₆-NDMA. The xylenes elute after NDMA but can co-elute if present in sufficient concentrations.

These compounds have minor ions at m/z 74 and will interfere with the m/z 74 of NDMA. The mass resolution required to differentiate them from NDMA is 2,285. Operation of the HRMS at 7,000 RP is sufficient for this differentiation.

With the present level of sample cleanup, the ratio of m/z 42:74 that is used as a criterion for positive identification of NDMA by LRMS is of limited utility because it is frequently outside of the $\pm 20\%$ window even in relatively clean samples such as drinking water or laboratory procedure blanks. In some cases, this is due to interferences at m/z 42. However, interferences can also be detected at m/z 74 or m/z 80. In these cases, the quantitation will be adversely affected. For LRMS to be a viable technique, a more rigorous sample cleanup (separation of neutrals from neutrals) and possibly better chromatography are necessary.

Single ion monitoring by LRMS does not afford sufficient selectivity for a positive identification of NDMA. Therefore, a protocol that utilizes a minimum number of sample preparation steps requires confirmation, or analysis by HRMS for positive identification and accurate quantitation.

A Turn-Key FTIR System for the Analysis of Gas Phase Polychlorinated Biphenyls

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Abstract

A turn-key gas phase polychlorinated biphenyl analysis system has been developed using Fourier transform infrared spectrometry, artificial intelligence assisted library searching and an automated infrared interpreter. Vapor phase infrared spectra of 37 PCB isomers have been measured and used to define the interpretation rules. The library search algorithm and the infrared interpreter take advantage of the portability, computing power and mass data storage capability of PC/DOS systems and is demonstrated to be a cost effective tool to ensure consistent data interpretation. This turn-key system allows for rapid identification of complex PCB mixtures with elucidation of contributing PCB congeners.

Introduction

An automated infrared interpreter (PAIRS ⁺) was developed on an IBM personal computer (PC) running under the Microsoft disk operating system (DOS). Details of this program and its application for the analysis of oils and greases is given elsewhere (1). Briefly stated, PAIRS ⁺ combines both artificial intelligence and library search capabilities to interpret the infrared spectra measured with a wide variety of spectrophotometers. The library search subprogram uses five main algorithms: absolute difference (AB); square of absolute difference (SQ); absolute difference of first derivative (AD); square of difference of first derivative (SD) and time domain cross correlation (FT). A sixth additional algorithm, the combined algorithm (CO) was developed combining the library search results from the first five algorithms resulting in a higher confidence level for the reported data.

An artificial intelligence approach to gas phase PCB analysis in PAIRS⁺ has been devised which can identify different types of chlorinated benzene rings based on the position, intensity and full width at half maximum (FWHM) of the infrared bands in the spectrum. These information have been obtained from the spectra of 37 PCB isomers that we have measured. With the combination of a suitable GC column, appropriate GC experimental conditions and this program it may be possible to identify all 209 PCB isomers which may be present in the sample.

Experimental

Solutions of 37 individual PCB isomers (Ultra Scientific, Wellington, CIL) were prepared in iso-octane with an initial concentration of between 100-150 ppm. Table 1 gives the list of these PCB isomers. Solutions were further concentrated if the S/N ratio of the infrared spectrum was less than 5. A Nicolet 5SX (Madison, WI) FT-IR spectrometer equipped with an air cooled nichrome wire source and a medium range cryogenic HgCdTe detector (cut-off frequency at about 600 cm⁻¹) was used to measure the GC/FT-IR spectra. The gas chromatograph used was a Hewlett-Packard 5890 (Palo Alto, CA) equipped with an on column injector, a wide bore column (cross-linked methyl Silicon Gum, 25 m x 0.32 mm i.d. x 1.0 micrometer film thickness) and a flame ionization detector. The starting temperature on GC for di-, tri-, and tetra- isomers was set at 200 °C and for penta- and hexa- isomers it was set at 210 °C. An initial hold time of 0.5 minute on GC was used and temperature was ramped at a rate of 3 °C/min to 270 °C. The injector temperature was set at 250 °C. The dimensions of the light pipe used were 12 cm x

0.1 cm and was operated at 270 °C throughout the experiment. Interferograms were collected at a sampling rate of 50 kHz, obtaining infrared spectra with 8 cm⁻¹ resolution. The Gram-Schmidt orthogonalization algorithm (2) was used to reconstruct gas chromatograph from the interferograms and the infrared spectra.

Results and Discussion

The structure of each polychlorinated biphenyl consist of two benzene rings in which one to ten hydrogen atoms can be substituted by chlorine atoms. Substitution of these chlorines will give rise to eighteen (including a benzene group for PCBs with one unsubstituted ring) different types of chlorinated benzene rings. For example when one chlorine atom is present in the structure of the PCB, three different isomers i.e., 2-mono, 3-mono, and 4-mono biphenyls are possible. When two chlorines are present, 6 different isomers i.e., 2,3-di, 2,4-di, 2,5-di, 2,6-di, 3,4-di and 3,5-di are possible etc. These eighteen types of substitutions give rise to different infrared bands in the spectral region 600 to 2100 cm⁻¹. Figure 1 illustrates the spectra for three of the 37 isomers. Using the 37 spectra, the characteristic infrared bands for each type were identified. The eighteen types of substitution and the characteristic infrared bands are given in Table 2. For each infrared band the averaged normalized intensity and the averaged estimated FWHM are given in the parenthesis. Each PCB isomer is a binary combination of any of these eighteen groups. For some groups the characteristic bands overlap, but at least one band for each group can be identified which is unique to that group.

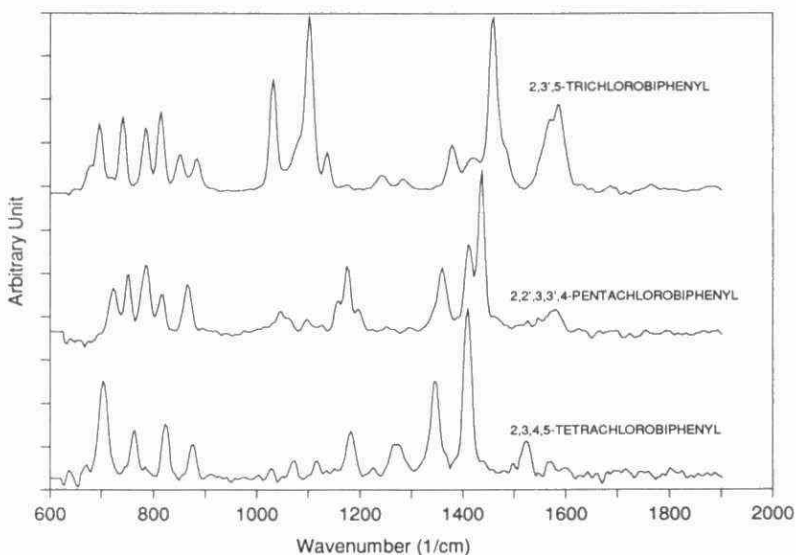


Fig. 1. Infrared gas phase spectrum of three PCB isomers.

Using the information given in Table 2, a program was written to interpret the data and identify the PCBs. This program requires three sets of information: 1) the spectral region of the bands pertinent to each group of PCB isomers where the algorithm will allow for the variations due to the types and the interaction between the two benzene rings, 2) a normalized intensity range from 1 to 10 where the bands will be considered as "weak" for the intensities in the range 1-2, "medium" between 3-6 and "strong" for the intensities between 7-10 and 3) an allowable range for the variation in the full width at half maximum (FWHM) of the bands

which will classify the bands as "sharp" for $\text{FWHM} < 15 \text{ cm}^{-1}$, "medium" for FWHM of $15\text{-}30 \text{ cm}^{-1}$ and "wide" for $\text{FWHM} > 30 \text{ cm}^{-1}$. These information have been obtained from the analysis of the 37 vapor phase spectra of PCBs (see Table 2) and can be modified and/or changed in the program as required.

Conclusion

Gas phase infrared spectra of 37 PCB isomers have been measured and analyzed. The information obtained from these spectra are used in the elucidation of PCBs. Since these 37 isomers represent all the possible eighteen different types of substituted benzene rings in the polychlorinated biphenyl structure, it may be used to identify all 209 possible PCB isomers. This technique offers the possibility of direct environmental analysis by remote sensing and the potential for simplified analytical methodology through elimination of clean-up steps.

References

1. M.J. Yang and P.W. Yang, Appl. Spectrosc., in press (1991).
2. L.V. Azarraga and D.A. Hanna, GIFTS, Athens GC/FT-IR Software User's Guide, US EPA/ERL, Athens, GA, 1979.

Table 1- List of PCB isomers measured

Dichlorobiphenyls

1) 2,2'

2) 2,4'

19) 2,3',4,4'

20) 2,3,4,5

21) 2',3,4,5

Trichlorobiphenyls

22) 2,3',4',5

23) 2,3',5,5'

24) 2,4,4',6

25) 2,3,5,6

26) 3,3',4,4'

3) 2,2',5

4) 2,3,4

5) 2',3,4

6) 2,3',5

7) 2,4,4'

8) 2,4,5

9) 2,4',5

Pentachlorobiphenyls

27) 2,2',3,3',4

28) 2,2',3,4,4'

29) 2,2',3',4,5

30) 2,2',3,4,5'

31) 2,2',4,5,6

32) 2,3,4,5,6

33) 2,3,4,4',5

34) 2',3,4,4',5

Tetrachlorobiphenyls

10) 2,2',3,4'

11) 2,2',4,4'

12) 2,2',3,5

13) 2,2',3,5'

14) 2,2',4,6

15) 2,2',5,5'

16) 2,2',5,6'

17) 2,3,3',4

18) 2,3,4,4'

Hexachlorobiphenyls

35) 2,2',3,3',4,4'

36) 2,2',3,3',4,5

37) 2,2',3,3',5,6

Table 2- Characteristic infrared bands for different types of substituted benzene rings.

Type of Substitution	Characteristic ir band(s)
2	751(8 ^a ,17 ^b), 1038(4,18)
3	740(5,15), 783(4,19)
4	823(5,22), 1012(3,16), 1094(8,19)
2,3	726(5,20), 1409(7,21)
2,4	821(6,18), 869(2,20), 1103(6,18)
2,5	815(6,18), 1097(8,24), 1376(3,20)
2,6	783(9,27)
3,4	1035(6,22), 1134(6,24)
3,5	806(7,23), 1099(6,15), 1562(10,11)
2,3,4	784(5, 21), 817(4,19), 1174(4,21), 1358(4, 23)
2,3,5	1034(8,23), 1095(9,30)
2,4,5	886(4,20), 1045(4,28), 1091(4,23), 1139(4,23)
2,4,6	838(8,18), 1543(6,23), 1577(7,26)
3,4,5	810(5,19), 876(2,19)
2,3,4,5	1179(4,23), 1342(6,23)
2,3,5,6	1062(8,21), 1388(10,31)
2,3,4,5,6	1323(7,27), 1377(6,19)
Benzene ring	699(7,22)

^a Intensity on the scale of 1 to 10, 1 being weakest and 10 being the strongest peak.

^b Full Width at Half Maximum (FWHM).

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